USING MULTI-ELEMENT STABLE ISOTOPE DILUTION TO QUANTIFY METAL REACTIVITY IN SOIL

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Abstract

Determining the total concentration of elements in soils seldom provides sufficient insight into trace metal bioavailability. However, measurement of 'isotopically exchangeable' metal can provide a better evaluation of metal reactivity and potential toxicity. Traditionally this requires the use of problematic radio-isotopes (e.g. ^{109}Cd (γ)). Fortunately, increasing access to Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in recent years has led to greater use of enriched stable isotopes of trace metals. The lability of heavy metals has been determined through a variety of approaches, including single and sequential extraction or predicted by geochemical models. In the present work, multi-element stable isotopes methods were developed for simultaneously determination of the labile pool of Fe, Zn, Cd and Pb using isotopic exchange principles. This included experimental and instrumental development for an accurate and precise determination of labile metal pool in soils. This approach was then validated by quantifying Zn, Cd and Pb in contaminated soils (Derbyshire; n = 8 and Weardale catchment; n = 246) and comparing the outcome results with common traditional extraction procedures. The variation of metal lability with soil characteristics was used to predict metal lability from the simple soil measurements using a multiple regression approach. In addition, E-values of Fe, Zn, Cd and Pb was used as input to WHAM(VI) ((Windermere Humic-Aqueous Model) to predict metal solubility, emphasising in the role of Fe under reducing conditions in this regard. The results showed that isotopic dilution is a robust mechanistic method for assessing the 'reactive' pool of multiple trace metals over a wide range of soil characteristics. The results showed a very wide range of metal reactivities (almost 1%-100%) for Zn, Cd and Pb that were consistent over a range of spike concentrations. Sub-micron forms of non-labile metal are perhaps most likely to occur in suspension either strongly bonded to humic/fulvic acids or occluded within $CaCO_3$ particles. It

appears that E-values have no consistent correspondence to any chemical extraction procedure. Nevertheless, the use of 0.43 M HNO_3 to extract labile metal in organic soils at pH < 6 appears justifiable - especially where humus is likely to be the principal adsorption surface. It is also important to acknowledge that extractions are not necessarily intended to estimate the entire reactive fraction. Thus, DTPA has been successfully applied as an empirical prediction of plant uptake but its extraction capacity is particularly limited in calcareous systems where it substantially underestimates the isotopically exchangeable metal pool. Speciation calculations showed that prediction of metal solubility was much better when the isotopically reactive metal pools were used as input to WHAM(VI). The soil samples that fitted best had pH values less than 4.0 and high organic matter contents reflecting the strength of the humic binding component of WHAM(VI) particularly in the case of Zn. The changes in metal solubility and lability under reducing conditions were mainly affected by pH. Moreover, the measurement of Fe^{2+} in the solution phase was considerably lower than that of the isotopically labile Fe^{2+} which calls into question the dependence on soluble Fe^{2+} to predict reductive dissolution of Fe-oxides. In addition, under reducing conditions the variables input of Fe to WHAM(VI) showed greatest effects on predicting metal solubility. It was found that Zn and Cd were affected only by Fe^{2+} competition for adsorption sites while predicted Pb solubility was more affected by loss of oxides than competition processes. The fractionation results, output from WHAM(VI), showed that a significant proportion of Pb was associated with Mn-oxides. Therefore, the calculation of loss of the adsorption site of Mn-oxides depending on Mn^{2+} measured in the solution phase did not improve the predicted Pb solubility where the model underestimate the adsorbed labile Mn as inference from Fe results.

Dedication

This work is dedicated to my mother and sisters for always pushing me to do my best, to the soul of my father for believing that I will and to my loving wife for encouraging me and bringing out the best in me.

I love you all

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1. INTRODUCTION

1.1 Overview

Metals in soils exist in a wide range of chemical forms, distributed between solid and solution phases. Metal ions associated with solid phases can differ widely in their 'chemical reactivity' or 'lability'. There are several factors that affect the degree of metal lability: (i) soil physiochemical properties (e.g. pH, redox potential, temperature etc), (ii) source of contaminant (natural or anthropogenic), (iii) the nature of soil solid constituents (e.g. organic matter, Fe/Mn oxides, clay minerals, carbonates and phosphates) and (iv) the reaction timescale being considered (Daviescolley et al., 1984, Ledin et al., 1996, Degryse et al., 2004, Hammer et al., 2006, Tack, 2010).

Thus, metal fractions in soils (Fig. 1.1) include:

(i) Inert forms (M_{Inert}) strongly incorporated into primary minerals, precipitated within secondary alteration products or possibly occluded within organic compounds.

(ii) Non-labile ($M_{Non\text{-}labile}$) forms of metal that are adsorbed to solid phase or suspended colloidal particles and are only exchangeable with the solution phase through a kinetically moderated process over a period of months or years.

(iii) Labile metal ions (M_{Labile}) which are instantly reactive in response to changes in equilibrium conditions and exist in the solution phase as free ions (M^{2+}), organic and inorganic complexes ($M\text{-}Cpx_{labile}$), and adsorbed to solid or suspended colloidal particulate surfaces.

Various methods have been developed to quantify metal solubility and fixation in soil. These include chemical extraction, speciation using multisurface models, diffusive gel membrane and isotopic dilution techniques (see Fig. 1.1). However,

soil is a complex system and measuring metal lability in such system needs an unambiguous technique. Multi-element stable isotope dilution should provide a resolution of the chemically reactive metal pool in the solution phase and in both the solid and nano-particulate phase. However, the most appropriate method to characterize metals in soils should depend on the specific aim of the study; combining different techniques may provide additional insights (Degryse et al., 2011). This chapter provides a brief overview of some widely used analytical methods for measuring metal lability, the overall objective of the current study and the structure of the thesis.

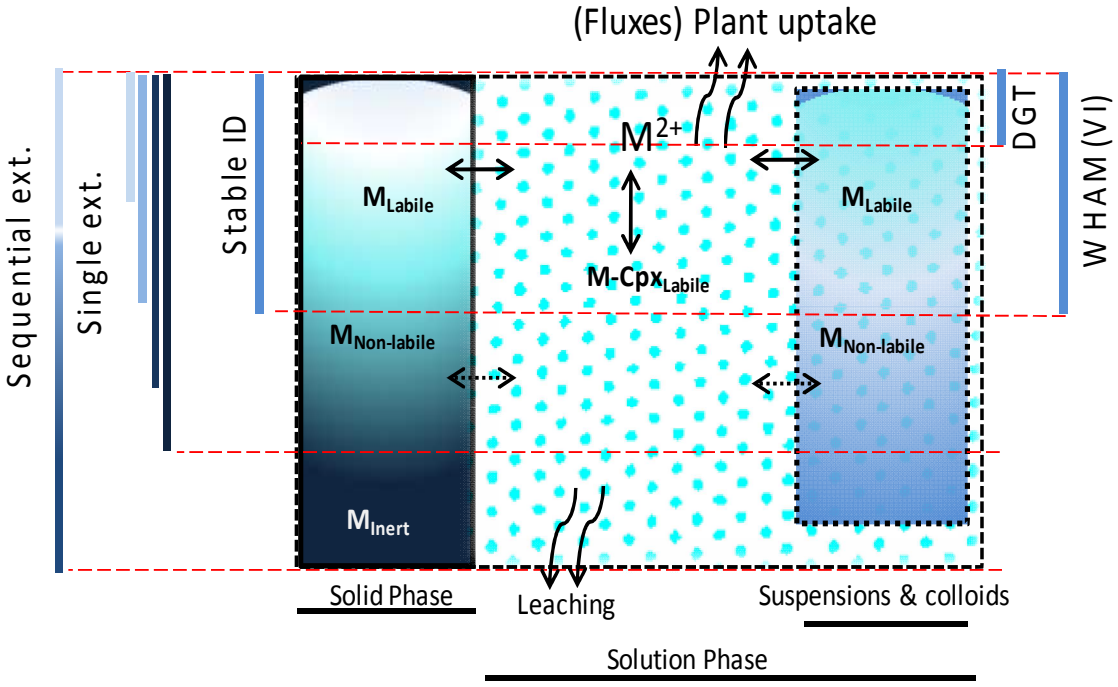

Fig. 1.1 Schematic representation of metal fractionation in soil-solution system revealed by different measurement and modelling approaches. Solid and broken reversible arrows represent the fast and slow kinetic reactions, respectively.

1.2 Approaches to estimating labile metal pool in soils

It is now widely agreed that the toxicity and the mobility of metal pollutants, such as Zn, Cd and Pb, depend strongly on their specific chemical forms and on

their binding state (precipitated with either primary or secondary minerals, complexed by organic ligands, and so on) (Gleyzes et al., 2002, Bäckström et al., 2004). Resolving metal fractionation and speciation in soils is vital to characterising the reactivity, bioavailability and mobility of metals in soils and may serve as an aid to the development of successful metal remediation methods (Ahnstrom and Parker, 2001). Several techniques to determine the reactive pools of trace elements in soils have been developed, including: single and sequential extraction procedures (SEPs) (Li and Thornton, 2001), methods based on isotopic exchange (Tongtavee et al., 2005) and the diffusive gradient in thin film technique (DGT) (Roulier et al., 2008). To characterize the chemical forms of metals in the solid phase of soils (Fig 1.1), SEPs and ID are are possibly the two most applied techniques (Young et al., 2005). Chemical fractionation methods have been widely used to allocate trace metals into operationally defined pools of putatively distinct biogeochemical forms, classically designated as 'exchangeable', 'carbonate', 'organic', 'hydrous oxide', and 'residual') (Tack and Verloo, 1995). Generally, increasingly aggressive reagents are used, in sequence, to extract increasingly refractory forms of the target metals (Ahnstrom and Parker, 2001). By contrast, ID is a less intrusive technique that allows differentiation of trace-element pools on the basis of the kinetics of exchange of the trace elements with the soil solid phase (Hamon et al., 2008) but is much less widely used compared to SEPs.

1.2.1 Single extraction methods

A wide range of methods using single extraction steps have been used to operationally define the fraction of metal that is potentially chemically reactive (Lindsay and Norvell, 1978, Gupta and Aten, 1993, Novozamsky et al., 1993, Ure, 1996, Young et al., 2000, Meers et al., 2007). Methods are based on the premise that labile metal is exhaustively solubilised using a single chemical extractant, whereas non-labile and inert metal is left in the solid phase. Several

parameters should be considered in selecting an extractant: the soil nature (Boekhold et al., 1993), the extractant concentration, solid/liquid ratio, equilibration time (Meers et al., 2007), temperature and shaking speed (Ure, 1996). For instance, in fairly acidic soils, extraction with dilute strong acid such as 0.43 M HNO_3 or acetic acid may provide a useful estimate of the labile metal pool in the soils (Tipping et al., 2003). However the same extraction (0.43 M HNO_3) may not be suitable in alkaline calcareous soils where non-labile metal occluded in carbonate may be attacked and solubilised by the acid (Degryse et al., 2009a).

Although more sophisticated analytical methods have been developed to estimate the labile metal pool in soil, single extraction methods are still favoured because they are simple to apply, cost-effective and provide satisfactory results in many cases. Single extractants can be broadly categorized into three groups: **neutral salts** (such as $CaCl_2$ and $NaNO_3$), **chelating reagents** (such as EDTA and DTPA) and **weak organic and diluted inorganic acids** (such as CH_3COOH and HNO_3, respectively) (Table 1.1).

1.2.1.1 Neutral salts

Neutral salt extractants are widely used to estimate the exchangeable metal pool in the soil (Table 1.1). Novozamsky et al. (1993) concluded that only 'weakly adsorbed' metal should be considered as 'plant-available'. They proposed the use of a 0.01 M $CaCl_2$ to extract bioavailable metal in soil and sediments. This was regarded as similar to the 'typical' ionic strength of the soil solution with Ca ions as the most dominant cations in the soil pore water (Houba et al., 1996, Houba et al., 2000). Moreover, the divalent cations promote coagulation of the colloidal material in the soil suspension compared to monovalent cations (Meers et al., 2007). However, using NO_3 as the counter ions does not promote dissolution of metal through complexation as in the case of chloride-based extractants (e.g. 1 M $CaCl_2$ used to extract Cd, Young et al., 2000).

Pueyo et al. (2004) reported that using NH_4NO_3 to extract the labile metal pool in soils may cause NH_3-metal complex and include additional release of metal. In a comparative study, NH_4NO_3 appeared to be the best estimator for plant-available metal (see Meers et al., 2007 and references therein). Moreover, Belotti (1998) showed a good correlation between the metal extracted by NH_4NO_3 and the fraction that is bioavailable to earthworm species. Extraction of Cd, Cu, Pb and Zn with 0.1 M $NaNO_3$ produced systematically lower concentrations compared to either 1.0M NH_4NO_3 or 0.01M $CaCl_2$ (Pueyo et al., 2004). This may be due to several factors: the specific cation competitiveness for adsorption, the soil pH and the type of the extracted metal and consequently the ability of the extractant to release competing cations from the soil matrix. In general, (Gommy et al., 1998) (Cited in Meers et al., 2007) stated that the competitiveness of the cations used in single neutral salt-extractants to extract the exchangeable metal fraction has been observed as the following order: Ba > Ca > Mg > NH_4 > K > Na.

In comparison with isotopically exchangeable metal, only Cd extractable with 1 M $CaCl_2$ has really been shown to provide comparable results (Young et al., 2000). 'Exchangeable' metal, that is metal ions largely bound by electrostatic attraction rather than chemical bonding, is always less than the isotopically exchangeable fraction in soils (Young et al., 2005). In studies that compare 'extraction/equilibration' of soil in dilute neutral salts with plant uptake it seems that the basis of the comparison lies somewhere between measurement of a 'labile pool' (a capacity term) and a simulation of the concentration of metal ions in soil pore water (an intensity term).

Table 1.1 List of the most common single extraction methods

Group	*Extractants (mol L^{-1})*	*References*
Neutral salts	$CaCl_2$ (1.00)	(Young et al., 2000)
	$CaCl_2$ (0.10)	(Novozamsky et al., 1993)
	$CaCl_2$ (0.01)	(Ure, 1996)
	$CaCl_2$ (0.05)	(Novozamsky et al., 1993)
	$Ca(NO_3)_2$ (0.10)	(Meers et al., 2007)
	$NaNO_3$ (0.10)	(Gupta and Aten, 1993)
	NH_4NO_3 (1.00)	(Novozamsky et al., 1993)
	$AlCl_3$ (0.30)	(Hughes and Noble, 1991)
	$BaCl_2$ (0.10)	(Juste and Solda, 1988)
	$Ba(NO_3)_2$ (0.1)	(Gooddy et al., 1995)
	$MgCl_2$ (0.1 - 0.02)	(Makino et al., 2006)
	KCl (0.1 - 0.02)	(Makino et al., 2006)
Chelating agents	EDTA (0.01)	(Novozamsky et al., 1993)
	EDTA (0.05)	(Ure, 1996)
	DTPA (0.005)	(Lindsay and Norvell, 1978)
Acid extraction	HNO_3 (0.43)	(Gooddy et al., 1995)
(inorganic & organic)	HNO_3 (2.00)	(Novozamsky et al., 1993)
	HNO_3 (0.10)	(Tipping et al., 2000)
	HNO_3 (0.50)	(Meers et al., 2007)
	HCl (0.1 – 1.0)	(Novozamsky et al., 1993)
	CH_3COOH (0.43)	(Ure, 1996)
	CH_3COOH (0.10)	(Ure et al., 1993)

1.2.1.2 Chelating agents

Chelating extraction solutions were originally used to assess the bioavailability of trace nutrient metals (Co, Cu, Zn) and later applied to determining potentially toxic metals such as Cd and Pb (Lindsay and Norvell, 1978, Novozamsky et al., 1993). Metals that are exchangeable, associated with organic matter (Laing, 2010), adsorbed on Fe/Mn oxides (Rauret, 1998) and bound to carbonates (Quevauviller, 1998) are potentially accessible to chelating agents. However, such extractents can also solubilise a proportion of the soil mineral phase ($CaCO_3$, MnO_2 etc), which may result in extraction of metals contained within the non-labile pool (Singh, 2007). Nevertheless extraction procedures using chelating agents have been developed and tested for several decades. The use of EDTA in particular now has the added credibility of 'accreditation' by the Institute for Reference Materials and Measurements (formally BCR) through the issue of certified reference materials (CRM) (Quevauviller, 1998, 2002 and references therein). Four soils were certified using EDTA (0.05 M at pH 7) as extractant: two amended sewage sludge soils (CRM 483 and 484), a calcareous soil (CRM 600) and an organic soil (CRM 700). The extraction solution was assumed to extract both humus-bound and carbonate-bound fractions of metals and it therefore was considered to be suitable for calcareous soils (Laing, 2010). Whether these fractions may also be regarded as 'labile' or 'bioavailable' is unclear.

A mixed NH_4OAc/EDTA (pH 5.5) originally developed by Lakanen and Ervio (1971) has been said to simulate the proportion of metal that is available to plants (Meers et al., 2007). However, the mixture may not be 'suitable' for all metals as there is some evidence that Pb and Zn could be precipitated by EDTA at pH 5.5, according to (Laing, 2010). A relatively weak solution of diethylenetriamine pentaacetic acid (DTPA) 0.005 M (pH 7.3) was thought to exclude effects involving carbonate dissolution (Lindsay and Norvell, 1978). The

DTPA extraction method is widely used, predominantly for the determination of extractable Cd, Cu, Fe and Mn (Laing, 2010). The DTPA method extracts less than the standard EDTA (and NH_4OAc + EDTA) method. This may arise partly from the higher pH value of the DTPA extractant (7.3 vs. 5.5), lower concentration (0.005M vs. 0.05M), lower affinity of the DTPA than EDTA (Meers et al., 2007) and lower liquid : solid ratio (2 : 1 vs. 5 : 1).

1.2.1.3 Inorganic and organic acids

Dilute strong acids (e.g. HNO_3), and non-complexing weak acids (e.g. CH_3COOH), are likely to partially dissolve metals from most soil fractions (exchangeable, carbonate, Fe- and Mn-oxides, humus; Rauret, (1998). Metal extractions using dilute nitric acid (0.43 M) have been used by several authors to estimate 'chemically reactive' metal (Gooddy et al., 1995, Temminghoff et al., 1997, Cances et al., 2003, Tipping et al., 2003, Groenenberg et al., 2010). This approach is thought to present some advantages in that there is no mobilization of metals due to complex formation with the anion ligand (Meers et al., 2007, Tack, 2010). Diluted hydrochloric acid (0.1 M) has been operationally defined as a 'moderate acid extractent' (Fiszman et al., 1984, Barreto et al., 2004). Several studies have suggested that extraction with cold 1 M HCl provides a measure of the labile metal fraction (Sutherland et al., 2004, Cook and Parker, 2006, Choi et al., 2011). Bulk soil extraction with LMW organic acids, such as acetic and citric acids (e.g. 0.1 M CH_3COOH), have been used to assess 'phytoavailability' of metals within the rhizosphere of plants (Laing, 2010). Some studies have suggested the use of mixed solutions of CH_3COOH, CH_2O_2, $C_6H_8O_7$ and $C_4H_6O_5$ at a ratio of 2:2:1:1; again, the intention is to mimic the soil solution composition within the rhizosphere (e.g. Wang et al., 2003).

1.2.2 Sequential Extraction procedures (SEPs)

Sequential extraction procedures (SEP) have a slightly different purpose compared to single extraction methods. The objective of an SEP is to audit the

various chemical fractions of metal expected to be present in soil, rather than simply discriminate between 'reactive' and 'non-reactive' forms. As SEPs are 'sequential', they typically use a series of chemical extractants with gradually increasing extraction power (Filgueiras et al., 2002). Many SEPs currently used are developments of the approach suggested by Tessier et al. (1979) but they vary in the number of fractions and reagents used. The Tessier method partitions elements into five operationally-defined geochemical fractions (Fig. 1.2) including exchangeable, carbonate (acid-soluble), Fe and Mn oxides (reducible), organically bound (oxidisable) and residual (inert).

The limitations of this procedure (and of other SEPs) have been addressed by several researchers (Martin et al., 1987, Salt et al., 1995, Tack and Verloo, 1995, Young et al., 2005). These include the problem of achieving complete and selective dissolution and recovery of trace metals from specific phases in soils and sediments. Recognised problems include the lack of specificity of the chemical reagents used for extraction and 're-adsorption' of trace metals during the process of extraction (e.g. Young et al., 2005). In spite of these inherent limitations, SEPs are still considered a useful technique for characterizing (fractionating) trace metals in soils and sediments (Tack and Verloo, 1995, Arunachalam et al., 1996) and, at worst, they provide a relative assessment of operationally defined soil metal fractions for comparative purposes. Several researchers have modified the Tessier procedure to obtain a more discrete dissolution of the target fractions in soils and sediments (Li et al., 1995, Arunachalam et al., 1996, Ahnstrom and Parker, 1999, Rigol et al., 1999, Li and Thornton, 2001, Brazauskiene et al., 2008).

Table 1.2 Some sequential extraction procedures used to fractionate metals in soils. Adapted from Filgueiras et al. (2002) and Singh (2007)

Approach	*Extraction Step (F)*						
	F1	F2	F3	F4	F5	F6	F7
Krishnamurti et al. (1995)	$Mg(NO_3)_2$	NaOAc	$Na_4P_2O_7$	$NH_2OH.HCl$	H_2O_2 / $Mg(NO_3)_2$	NH_4Ox	NH_4Ox / HOAc
Salbu et al. (1998)	H_2O	NH_4OAc pH 7	NH_4OAc pH 5	NH_2OH / HCl + 25%HOAc	H_2O_2 / HNO_3	HNO_3	
Ahnstrom and Parker (1999)	$Sr(NO_3)_2$	NaOAC	NaOCl pH 8.5	$H_2C_2O_4$	HNO_3 + HCl		
Tessier et al. (1979)	$MgCl_2$	NaOAc	NH_2OH / HCl + HOAc	H_2O_2 / HNO_3	HF / $HClO_4$		
Edwards et al. (1999)	NH_4OAc pH 7	H_2O_2 pH 2	HNO_3				
Ure et al. (1993) (BCR)	HOAc	$NH_2OH.HCl$	H_2O_2/ NH_4OAc				

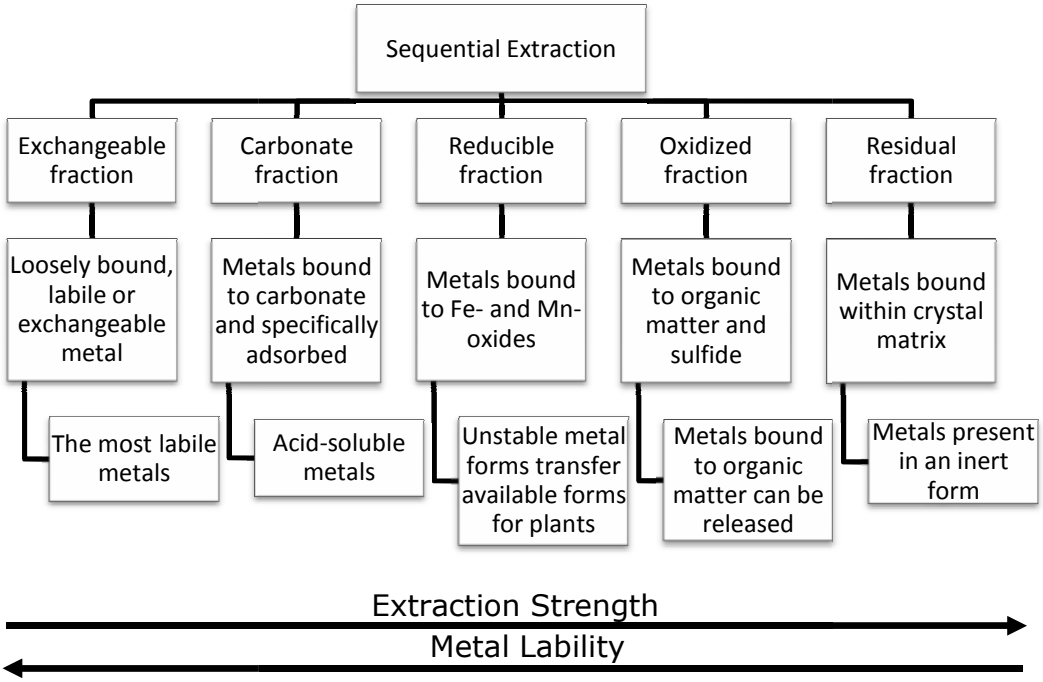

Figure 1.2 Overview of the five fractions of the Tessier SEP (Tessier et al., 1979) for metals fractionation in soil; the solid arrows show the relationship between the metal lability and the extraction strength in each fraction. Adapted from Filgueir et al. (2002) and Dean (2003 pp. 85).

1.2.3 Isotopic Dilution (ID) technique

Before reviewing isotopic dilution methods for estimating the labile metal content of soils, there are two terms that should be defined. The *E- value* is the metal pool which is isotopically exchangeable with the solution phase in a soil suspension; the *L-value* is the metal pool which is accessible to a plant growing in isotopically labelled soil (Midwood, 2007, Hamon et al., 2008) (Fig 1.3).

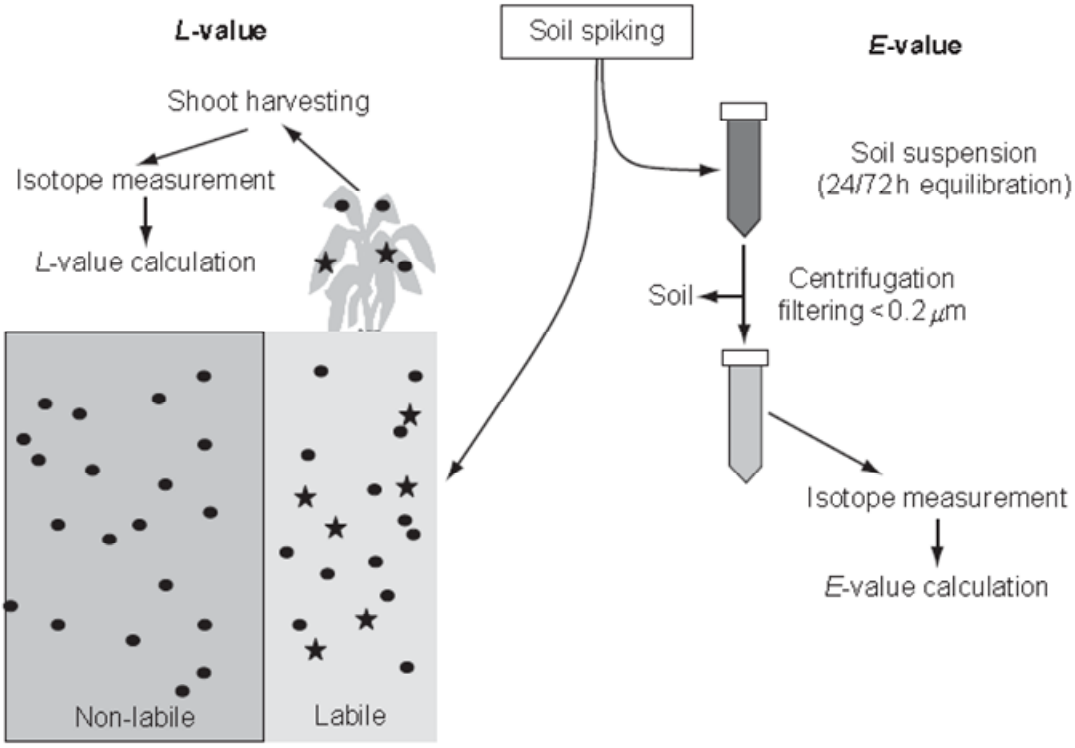

Figure 1.3 Schematic representation of the measurement of E- and L-values: in the L-value procedure, star and dot symbols represent the added isotope and the native metal respectively (Adapted from Hamon et al., 2008 pp 295).

Measurement of E- and L-values has employed radioactive isotopes for a limited range of elements. In recent years the range of applications has expanded to include Zn and Cd (Hamon et al., 1997, Young et al., 2000), Se (Goodson et al.,

2003), Ni (Echevarria et al., 2006), Cu (Lombi et al., 2003) and As (Tye et al., 2002). These studies all used radioactive isotopes as tracers, the analysis of which requires not only specific activity to be measured but also the total metal content of each sample. By contrast, Gabler et al. (1999) with Pb, Cd, Zn and Cu, Ahnstrom and Parker (2001) with Cd and Ayoub et al.(2003) with Cd and Zn used stable isotopes as tracers, assayed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). More recently, the greater availability of ICP-MS (Prohaska et al., 2005), has led to the wider use of enriched stable metal isotopes for environmental isotope dilution studies (Degryse et al., 2007, Gabler et al., 2007, Collins and Waite, 2009, Sterckeman et al., 2009, Atkinson et al., 2011, Huang et al., 2011). The use of stable isotopes permits the study of elements for which suitable radioisotopes are either not readily available or present logistical or safety problems. For instance, ^{210}Pb is not readily accessible to many research labs due to the exposure hazards associated with alpha radiation while ^{64}Cu has such a short half-life (12.4 hours) that handling is difficult (Lombi et al., 2003) and the range of possible applications quite limited. For Cu, *stable* isotope dilution may also allow assessment of the phytoavailable Cu pool (L-values) and longer term metal fixation studies, which are not possible with the short half life of ^{64}Cu. Stable isotopes also offer the advantage of allowing in-field isotopic experiments, since the environmental concerns regarding the fate of radioisotopes minimizes their utility in the field (Hamon et al., 2008).

1.2.3.1 Isotopic dilution principle

The E-value, or isotopically exchangeable metal content, of a soil is measured by adding an isotope tracer to a soil suspension, where the liquid phase is either water or a neutral salt solution such as diluted $Ca(NO_3)_2$ (Midwood, 2007) (Fig. 1.3). Typically, a soil will be initially suspended in the electrolyte for a specific pre-equilibration time. Then the suspension will be spiked with a stable isotope

which is allowed to equilibrate for a second specified period. The added isotope mixes only with the chemically reactive pool, not with inaccessible forms of metal. Supernatant solution will then be separated by centrifuge and filtration and the isotopic abundances of the target trace element will be determined by ICP-MS. Similarly, determination of L-values again involves the addition of an isotope to soil and thorough mixing with the native reactive metal pool. A plant is subsequently grown, usually from seed, and by measuring the isotopic composition of the metal in the plant tissue, the size of the labile pool available to the plant in the soil may be estimated (Fig. 1.3). Effectively, L-value is an estimation of the same labile pool measured in an E-value experiment, but the labile pool of metal is 'sampled' using a plant (Young et al., 2007, Hamon et al., 2008).

1.2.3.2 Factors affecting the ID experiments

i. Experimental cost: Purchase of stable isotopes and instrumental running costs

There are number of suppliers across the world from which stable isotopes can be purchased. A list of current suppliers can be found on the pages of the discussion group Isogeochem (www.isogeochem.wikispaces.com). A key consideration when working with stable isotope is the cost which varies between suppliers and depends on the degree of enrichment and the chemical form of the isotope. The isotopes can be expensive and individual experiments (especially field trials) may consume significant quantities so that care needs to be exercised when planning each experiment. In addition to the cost of the isotopes, analysis costs can also be considerable. For most laboratories, an ICP-MS is a sizeable money investment with high annual running costs. Moreover, the form of stable isotope should be considered when planning experiments. A water soluble salt form, for example, may be more appropriate for plant and soil

studies than the pure metallic form, which may have to be acid solubilised before use.

ii. Working spike solution: the choice of isotope and enrichment level

When a stable isotope is used for the determination of an *E*- or *L-value*, the amount of enriched isotope solution (spike) added to the system must be sufficiently large to cause a quantifiable change in the isotopic ratio of the spiked soil in comparison to the natural isotopic ratio of the system (Midwood, 2007). However, it is also important not to perturb the natural metal dynamics of the soil by adding large amounts of the isotope tracer. Tongtavee et al., (2005) followed the approach of Gabler et al. (1999) who determined the amounts of isotopically exchangeable Pb in soils using a ^{207}Pb spike of 10^{16} atom g^{-1} soil. Therefore, Tongtavee et al (2005) used 60 µl of an enriched ^{207}Pb solution (^{207}Pb = 94.6%, ^{208}Pb = 2.9%), which was spiked into the equilibrated soil suspension at a rate of 2.148 x 10^{16} atom g^{-1} soil of ^{207}Pb. Nolan et al. (2004) added ^{65}Cu equivalent to approximately 1% of total Cu in soil, the same method was adopted by Ahnstrom and Parker (2001) using ^{111}Cd. With respect to Pb, Degryse et al. (2007) used ^{208}Pb added in a relatively large amount (between 4 and 12% of the total Pb content). Ayoub et al. (2003) measured E values for Cd and Zn in two soils. The Great Billings soil, a contaminated soil, was spiked with 0.6 µg ^{111}Cd and 4.8 µg ^{67}Zn, while the Countesswells soil, with only background levels of trace metals, was spiked with 0.01 µg ^{111}Cd and 0.05 µg ^{67}Zn. The authors attempted to alter the $^{114}Cd/^{111}Cd$ ratio from 2.22 (natural abundance) to 1.96, and the $^{66}Zn/^{67}Zn$ ratio from 6.76 (natural abundance) to 6.10. The amount of spike added was based on the amount of Zn and Cd extracted by 0.43 M CH_3COOH as a preliminary estimate of the labile metal pools.

The isotopic natural abundance is a key consideration in selecting which isotope and the amount of spike that should be used. A low natural abundance of the spike isotope means that lower amounts of spike are required to produce a

measureable difference in isotopic abundance (Hamon et al., 2008, Atkinson et al., 2011). For example, Atkinson et al. (2011) and Degryse et al. (2007) used ^{204}Pb (1.4 % IA) added at ~2.70 times the native ^{204}Pb content and ^{208}Pb (52.4 % IA) at ~ 4.0 times the native ^{208}Pb content to determine Pb lability in soils, respectively. However, it is obviously vital that adequate spike is used to ensure the enrichment level remains measurable throughout the experiment (Midwood, 2007) in the case of kinetic studies.

iii. Equilibration time

There are two equilibration times to consider in measuring the *E-value*: pre-equilibration (before adding of the isotope) and post-equilibration times (after adding of the isotope). A period of pre-equilibration may reduce the need for extended contact between the isotope and soil while the native metal achieves solubility equilibrium and also for micro-aggregates to fully wet up (Young et al., 2007). Many different pre-equilibration times have been employed, including 5 days (Young et al., 2000), 3 days (Tongtavee et al., 2005), 24 h (Smolders et al., 1999, Nolan et al., 2004), 18 h (Ayoub et al., 2003), and zero (Pandeya et al., 1998). Hamon et al.,(2008) suggested that a post-equilibration time of 1 to 3 days is sufficient for isotopic equilibrium to be achieved. Similarly, Young et al. (2000) reported that after 48 h isotope equilibration time, very little change was seen in Cd *E*-values and suggested 2 days as a standard time for post-equilibration. Other authors have found that after 2-3 days the activity in solution stabilizes and remains almost constant for several days/weeks (Tongtavee et al., 2005, Oliver et al., 2006). This indicates that, although the selection of the post-equilibration time is purely an operational decision, in practice most published data suggest that a reasonably distinct labile pool is distinguishable when a 2-3 days of post-equilibration time is used (Young et al., 2000).

iv. Composition of suspending matrix

An ideal suspending matrix should solubilise a measurable amount of the target metal, and allow rapid isotopic equilibration, without attacking the non-labile metal pool. Various suspending electrolytes have been employed in the determination of E-values. Normally, either deionised water or a dilute electrolyte solution such as 0.1 M or 0.01 M $CaCl_2$ or $Ca(NO_3)_2$ (Young et al., 2000; Smolders et al., 1999) have been used. There are some advantages in using a neutral salt solution. In contrast to deionised water, the target metal concentration will be increased in the extracts as a result of displacement from adsorption sites. This is especially true for Cd, and other metals, if $CaCl_2$ is used to mimic the Ca concentration in the soil solution. The Cd concentration in solution is substantially enhanced by the complexation of Cd by Cl (Young et al., 2000). These characteristics are of importance when the concentrations of the measured metal in solution are close to their analytical detection limits because increased concentrations usually translate to more accurate measurements. Furthermore, salt solutions also offer another advantage by flocculating colloids from suspension which facilitates filtration of the samples and diminishes the potential for colloidal interferences (Hamon et al., 2008).

However, it is also important to ensure that the suspending electrolyte does not cause precipitation of the target metal which would result in an underestimation of the labile pool or cause dissolution of sorption surfaces such as Fe/Mn hydrous oxides which would increase the apparent E-value. For example if the aim is to evaluate *E-values* for Ag or As, it would be better to avoid use of salt solutions containing Cl or Ca, respectively. This is due to the potential risk of precipitation of AgCl or insoluble calcium arsenate salts. Equally the use of complexing agents, such as EDTA, while increasing the solubility of the labile metal, run the risk of releasing otherwise non-labile metal through solubilisation of $CaCO_3$ or hydrous oxides (Atkinson et al., 2011).

v. Separating the supernatant from the solid phase

The last step before introducing the sample to the instrument (ICP-MS) is isolation of the supernatant phase by centrifugation and filtration. The solution phase may contain suspended colloidal particles that might carry non-labile forms of the analyte metal. Only removing the colloidal particles, including submicron suspended particulates, can guarantee an authentic measurable E-value. Nolan et al. (2004) and Tongtavee et al. (2005) filtered the solution through a 0.45 μm cellulose acetate membrane after separating the supernatants from the solid phase by centrifuging. Ayoub et al. (2003) employed two filtration steps, using Whatman No. 542 filter paper and then using Millipore 0.20 μm pore size membrane filters. Colloidal particles in soil solutions can result in overestimation of the soil reactive metal pool if they contain analyte metal ions that are inaccessible to the spiked isotope. This phenomenon was reported by Hamon and McLaughlin (2002) and Lombi et al. (2003). They studied the apparent effect of suspended submicron colloidal particulates (<0.1 μm) on isotopically exchangeable Cd, Zn and Cu. Thus, after filtration of the soil with 0.45 or 0.2 μm filters, an ion exchange resin (Chelex-100 resin) is used to adsorb a sample of the isotopically exchangeable pool. The resin is then washed and eluted with acid to measure the isotopic abundance of the spike isotope and hence the true E-value of the soil.

1.2.4 Diffusive Gradients in Thin Films (DGT)

Diffusive gradient in thin film (DGT) is a widely used technique used to measure several aspects of trace metal dynamics in soils and surface waters. It was originally developed for application to natural waters for *in situ* measurement of metal speciation (Davison and Zhang, 1994). The DGT device is a simple plastic container with three layers: (i) a resin layer (Chelex-100) overlain by (ii) a diffusion gel (pure gel) and (iii) a protective filter (Fig. 1.4).

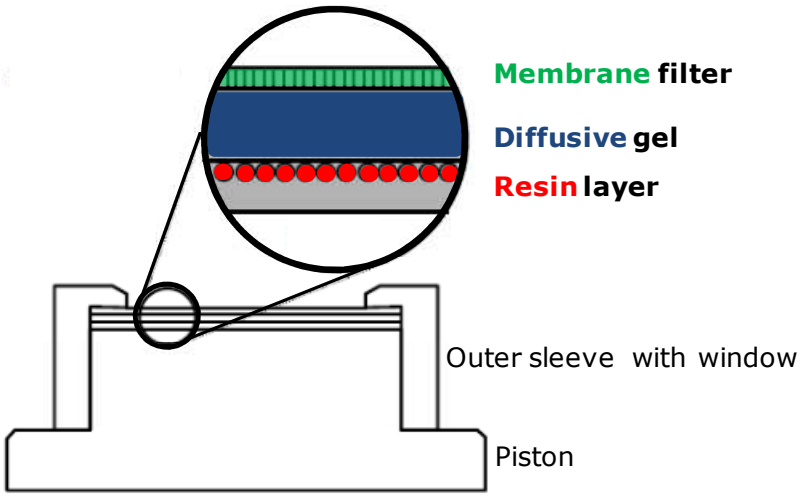

Figure 1.4 Schematic cross-section of DGT device (adapted from Dahlqvist et al., 2002).

The resin layer (Chelex-100) in the DGT (Fig. 1.4) serves as a sink, inducing a flux of metal ions from the soil, through the diffusion gel to the resin. The metal uptake by the resin depends on both the initial solution concentration and the rate of metal re-supply from solid phase to solution (Davison et al., 2000). DGT addresses two shortcomings associated with conventional methods used to predict bioavailable (plant-available) metal. Firstly, trying to predict a 'capacity' term through chemical extraction of soil inevitably changes the nature of the soil to an unknown degree. By contrast DGT can be used to assay the labile metal reservoir by inducing desorption from soil in response to lower metal concentration in solution – thus mimicking uptake by plant roots (Zhang et al., 2001). Secondly, measuring an 'intensity' term in isolation (e.g. soil pore solution concentration) ignores metal re-supply from the solid phase to solution phase (Hooda and Zhang, 2008). The action of DGT accounts specifically for re-supply, which will vary between soils, and enables the derivation of a unique characteristic of the soil 'C_{DGT}' which is equivalent to the time average concentration of the metal at the interface of the soil and the diffusive gel. A full description of the principles of the DGT device and calculation of the 'DGT concentration' (C_{DGT}) can be found elsewhere (Zhang and Davison, 1995, Zhang and Young, 2005).

The labile metal fractions sampled by DGT have been evaluated under a wide variety of laboratory and field conditions ranging from natural water to *in situ* analysis of sediments and soils. The range of elements studied have included Al, Cd, Co, Cu, Fe, Ni, Pb, Zn, U, Hg, Ca and Mg (Davison and Zhang, 1994, Davison et al., 2000, Dahlqvist et al., 2002, Ernstberger et al., 2002, Degryse et al., 2003b, Docekalova and Divis, 2005, Ernstberger et al., 2005, Zhang et al., 2006, Dunn et al., 2007, Vandenhove et al., 2007, Hooda and Zhang, 2008, Degryse et al., 2009b, Zhang et al., 2011) and, more recently, P and As (Mojsilovic et al., 2011). There are several limitations to the technique including: (i) incomplete recovery by elution of metals from the resin layer (Zhang et al., 1995); (ii) low adsorption capacity in the resin layer which limits the deployment time of the DGT in some cases (Zhang et al., 1995); (iii) problems associated with biofouling (Gimpel et al., 2001); (iv) competition effects of Ca on the resin layer (Chelex-100) which can affect Cd and Cu sorption at high Ca concentrations (0.01M) (Zhang et al., 1995, Gimpel et al., 2001); (v) effects arising from changes in pH (Gimpel et al., 2001), temperature (Zhang et al., 1995) and ionic strength (Peters et al., 2003, Warnken et al., 2005) on the labile metal species sorbed by the Chelex-100 resin. There are also uncertainties associated with the derivation of underlying characteristics, such as desorption rate constant and the labile metal pool, solely from the accumulated metal in the resin phase.

1.2.5 Windermere Humic Aqueous Model - WHAM(VI)

The geochemical speciation model WHAM(VI) (Windermere Humic Aqueous Model, version 6 (Tipping, 1998)) considers metal interactions with humic and fulvic acid. The current version of WHAM(VI) is simply a combination of humic ion-binding model VI (Tipping, 1998), an inorganic speciation model and sub-models to describe the binding of ions to Fe, Mn and Al hydrous oxides (Lofts and Tipping, 1998) and to a simple (alumino-silicate clay) cation exchanger. The

software package of WHAM(VI) is currently available at the Center of Ecology and Hydrology (CEH) website (http://www.ceh.ac.uk/products/software/wham/ - accessed: 11th November 2011).

Humic Ion-Binding model VI is the most important part of the overall model. Humic and fulvic acids are assumed to be rigid spheres, of uniform size, with four relatively stronger acid groups (Type A; carboxyl groups) and four weaker acid groups (Type B; hydroxyl groups). There are twice as many type A groups and within each type (A or B) the four groups are assumed to be present in equal amounts. Both A and B sub-groups have four evenly spaced intrinsic pK_a (pK_{int}) values. Metal cations compete for binding to each of the eight sites and the range of binding strengths is expanded by assuming formation of bidentate- and tridentate complexes (Tipping, 1998). The current version of WHAM will shortly be superseded by version 7, WHAM(VII) (announced on CEH website to be available for users by Autumn 2011). Humic Ion-Binding model VII includes a simpler description of multidentate metal binding sites than the current Model (Tipping et al., 2011). Moreover, in WHAM(VII) the users will be able to assess the model performance by incorporating uncertainty into the model predictions (Lofts and Tipping, 2011).

WHAM has been successfully applied in numerous studies, addressing geochemical topics such as acidification of soils and surface waters (Tipping et al., 1995, Tipping, 2005, Cooke et al., 2008), ground water chemistry (Christensen et al., 1999), Fe and Mn geochemistry (Tipping et al., 2002, Neal et al., 2008), organic matter solubility in soils (Tipping and Woof, 1991, Lofts et al., 2001), catchment scale ion transport (Tipping et al., 2006a, Tipping et al., 2006b), cation competition for binding (Mandal et al., 2000), the influence of competing ligands etc (Guthrie et al., 2005). Nevertheless, there are inevitably some limitations to the predictive ability of WHAM(VI). These include the following issues.

a. 'Active humic substances' (AHS). There is no satisfactory approach to determine the amount of AHS content in soils. Few studies resort to extracting and measuring the humic and fulvic acid content directly. Therefore, various assumptions have been proposed to provide an estimate of AHS content. Weng et al. (2002) assessed two separate assumptions - that dissolved organic matter (DOM) included: (1) 30% humic acid, 30% fulvic acid and 40% inert fraction; (2) 65% fulvic acid and 35% inert fraction. They found that there were no significant differences between the two assumptions in the predictions produced by WHAM(VI). Other researchers have assumed that DOM contains 50% C and that 65% (Cheng et al., 2005) or 70% (Balistrieri and Blank, 2008) of the DOM consists of AHS.

b. The type of Fe-oxyhydroxides (hematite, goethite, ferrihydrite or hydrous ferric oxide) and their specific surface area. In neutral soil pH, calcareous soils and enriched Fe-oxyhydroxides soils, the characterization of the reactive surface on oxyhydroxides is a sensitive parameter for modelling metal solubility (Buekers et al., 2008a, 2008b). Buekers (2007) used WHAM(VI) to predict the metal solubility of Cd, Zn and Ni in 28 soils assuming different types of pure Fe-oxyhydroxides and using their measured specific surface area as input variables. He found that the measured specific surface area of the oxyhydroxides caused overestimation of the solubility of Zn, Cd and Ni by factors of 4–7. Predictions were improved by increasing the specific surface area of Fe-oxyhydroxides from measured values for synthetic systems to a value of 600 $m^2\ g^{-1}$ which is equal to the default value used in WHAM. Buekers

concluded that the binding site capacity is more critical than differences in surface properties between Fe-oxyhydroxides.

c. Defining the reactive metal in soil. The soil metal 'content' should include only concentrations of 'reactive' metal rather than of total metal as the pool of metals that react with the surfaces (Weng et al., 2002). A wide range of chemical extractants have been used to estimate the reactive metal pool in the soils, such as 0.43 M HNO_3, 0.1 M HCl, and 0.05 M EDTA (Romkens et al., 2009). Reactive metal content can also be measured using isotopic dilution. This method is conceptually most attractive because it determines the concentrations of metals that instantaneously react with the solution. In comparative studies, the fraction of isotopic exchangeable metal was found to be smaller in pH-neutral and calcareous soils (Tye et al., 2003, Buekers et al., 2008a, Buekers et al., 2008b, Buekers et al., 2007), suggesting that the distinction between reactive and total metal concentrations may be critical for modelling trace metal solubility in these soils.

d. Alternative adsorption constituents, such as carbonates and hydroxy-interlayered minerals phases. Carbonates play an important role in sorption of numerous trace elements in soils. For example, Ahmed et al. (2008) used ID to study the fixation of Cd^{2+} to pure calcite. They found that after 210 d, 87% of total Cd was isotopically non-exchangeable while 9% remained as 'labile sorbed' and 4% was in the solution phase. Degryse et al. (2011) studied the isotopically labile pool of Zn in 51 contaminated soils previously characterised by Zn K-edge X-ray absorption fine structure (XAFS) spectroscopy. They found small proportions of isotopically labile Zn were observed for

acidic soils (pH ≤ 6.5) with a large fraction of Zn adsorbed by 'hydroxy-interlayered' alumino-silicate clay minerals.

e. WHAM also, cannot simulate precipitation and dissolution of solids or redox reactions (Gonçalves, 2005).

1.3 Objectives and thesis structure

The primary aim of this project is to develop and evaluate the measurement of Zn, Cd and Pb lability using multi-element stable isotope technique in a wide range of soils. Part of the testing procedure is to evaluate the usefulness of isotopically exchangeable metal as an input variable to WHAM. The underlying objective is to produce a widely applicable, simple and cost effective analytical approach to determining 'reactive' metal in soils. The thesis is organized in the following sequence.

Chapter 2 presents an outline of the analytical and procedural methods used throughout the investigation. This includes a range of general analytical methods, such as pH, organic matter content, total metal content, metal extraction procedures and a description of the input file to WHAM(VI). Moreover, the development of a multi-element stable isotope dilution technique for Zn, Cd and Pb was a key step in the current study including optimizing both the instrumental and experimental conditions to maximise accuracy and precision in the determination of E-value while minimising the cost and time required to undertake the assay. An initial step was to apply a preliminary isotopic dilution (ID) method to suspensions of isotopically spiked Amberlite IR-120 with the expectation of 100% recovery, as both a procedural and instrumental test.

In Chapter 3, the ID method is applied to eight contaminated soils which varied in their characteristics (pH, organic matter content, metal and carbonate content) collected from two historical Pb/Zn mining areas in Derbyshire to measure E-values for Zn, Cd and Pb. The effects of spike concentrations, pH and

the presence of submicron colloidal particles on apparent E-value were evaluated. The E-values are compared with traditional chemical extraction including single (DTPA, EDTA and HNO_3) and sequential extraction procedures.

Chapter 4 is a study of the dynamics of Zn, Cd and Pb in soils distributed across a partially contaminated catchment in Weardale, North Pennines. Variation in both soluble and labile Zn, Cd and Pb in over 250 soils was investigated. From the resulting dataset, predictive algorithms are developed from the soil properties to estimate lability of Zn, Cd and Pb. Moreover, the capability of WHAM(VI) to predict metal solubility utilizing the isotopically exchangeable Zn, Cd and Pb as input variables is tested.

The effects of redox potential on metal lability and solubility are presented in Chapter 5 where multi-element stable isotope dilution is used to follow changes in metal lability under fluctuating, and constant, redox potentials. Moreover, following the change in Fe-oxide content due to reductive dissolution was investigated using ID with enriched ^{57}Fe. The dual role of Fe as a competitor and as a source of solid phase adsorption sites was studied using WHAM(VI) parameterized under different scenarios utilizing isotopically exchangeable Fe, Zn, Cd and Pb.

General conclusions of the key findings of the thesis are presented in Chapter 6.

2. CHAPTER TWO: GENERAL MATERIALS AND METHODS

This chapter represent general materials and methods and the steps for developing the multi-element stable isotope dilution technique. Method development involved optimising both experimental conditions and instrumental parameters to maximise accuracy and precision in the determination of E-value while minimising cost and maximising efficiency of the assay.

2.1 BASIC LABORATORY PROCEDURES

2.1.1 Reagents

All chemicals used were of Trace Analysis Grade (TAG) or Analar (AR) quality; Milli-Q water (≥ 18.2 MΩ cm^{-1}) was used throughout. Prior to use, all glassware, bottles and centrifuge tubes were thoroughly cleaned with phosphate-free and biodegradable detergent (Decon) then soaked in 2 % (v/v) TAG HNO_3 and rinsed several times with deionised water. Enriched stable isotopes, ^{57}Fe (≥ 99.9% ^{57}Fe), ^{70}Zn (≥ 99.9% ^{70}Zn), ^{108}Cd (≥ 70.3% ^{108}Cd) and ^{204}Pb (≥ 99.4% ^{204}Pb), were purchased from ISOFLEX USA as metal foil samples. The isotope foils were initially dissolved in HNO_3 and then diluted with Milli-Q water to give a stock solution with concentrations of 890, 154, 192 and 380 mg L^{-1} for ^{57}Fe, ^{70}Zn, ^{108}Cd and ^{204}Pb respectively, in ~ 5% HNO_3. Working 'spike solutions' for isotopic exchange experiments were made up freshly from this stock.

2.1.2 Soil chemical characteristics

Soil pH was determined in soil:water suspensions (1:2.5 ratio) using a pH meter and combined glass electrode (Ag/AgCl) (Model pH 209, HANNA Instruments, Bedford, UK). Loss on ignition (LOI) was measured by heating at 550 ºC in a muffle furnace for eight hours (Heiri et al., 2001). Total soil carbonate content was measured by the Collins' Calcimeter method (Piper, 1954). For multi-element analysis, soil subsamples were ground in an agate Planetary Ball Mill (Model PM400; Retch GnbH & Co., Germany). Approximately 200 mg of finely

ground soil was digested using 2.5 mL hydrofluoric acid (HF; 40 % AR), 2.0 mL HNO_3 (TAG), 1.0 mL $HClO_4$ (AR) and 2.5 mL H_2O in a 48-place Teflon-coated graphite block digester (Model A3, Analysco Ltd, UK) with PFA digestion vials using program one and two; the temperature sequence for program one and two is presented in Table 2.1. Multi-element analysis was undertaken by ICP-MS (Model X-Series^{II}, Thermo-Fisher Scientific, Bremen, Germany) in 'collision cell mode' (7% hydrogen in helium) to reduce polyatomic interferences. Samples were introduced from an autosampler (Cetac ASX-520 with 4 x 60-place sample racks) through a concentric glass venturi nebuliser (Thermo-Fisher Scientific; 1 mL min^{-1}) and Peltier-cooled spray chamber (3^{o}C). Internal standards introduced to the sample stream via a T-piece included Sc (50 µg L^{-1}), Rh (10 µg L^{-1}) and Ir (5 µg L^{-1}) in 2% TAG HNO_3. External multi-element calibration standards (Claritas-PPT grade CLMS-2, Certiprep) included Al, As, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Rb, Se, Sr, U, V and Zn in the concentration range 0.00 - 100 µg L^{-1}. Each digestion batch included three procedural blanks and three samples of the certified reference material NIST SRM 2711 (Montana soil) for quality assurance. Available phosphate was extracted with 0.5 M sodium bicarbonate ($NaHCO_3$) solution at pH 8.5 (Olsen et al., 1954) and assayed using a variation on the phosphomolybdate method (Drummond and Maher, 1995) by measuring absorbance in a 1 cm cell at 880 nm (CE 1011 spectrophotometer, Cecil Instruments). Total organic and inorganic carbon (TOC and TIC) in filtered solution were determined using a Shimadzu TOC-V CPH/CPN Total Organic Carbon Analyser (Model TNM-1) using N_2 as the carrier gas. Amorphous Fe and Mn oxide content was measured according to the adjusted methods described by Anschutz et al. (1998) using a mix regents of sodium citrate (50 g), sodium bicarbonate (50 g) and ascorbic acid (20 g) at pH 8.0 into 1 litter of MilliQ water. One gram of air dried soil was mixed with 20 mL of reagent prepared, shaken for 24 h, and filtered (< 0.2 µm). The soil aliquot was acidified in 1-in-10 with 0.1 M HCl prior to analysis by ICP-MS.

Table 2.1 The temperature and dwell time sequence for program one and two using block digester.

Step NO.	Program 1		Program 2	
	Temperature (° C)	Dwell Time (hrs)	Temperature (° C)	Dwell Time (hrs)
1	30	0.50	120	1.00
2	50	1.00	140	3.00
3	80	14.0	160	4.00
4	30	Till end	50	Till end

2.2 Multi-element stable isotope dilution

2.2.1 Preliminary test of Isotope Dilution (ID) methodology

Preliminary experiments were carried out using Na saturated Amberlite IR-120 resin (Fisher Scientific Ltd, UK), enriched with Cd, Zn and Pb, with the expectation of measuring an E-value equivalent to 100% of the added metal. Resin samples (0.5 g) were equilibrated in Oak Ridge polypropylene centrifuge tubes with 30 mL 0.01 M $Ca(NO_3)_2$ containing aliquots of Zn, Cd and Pb. The concentrations added were gauged from the results obtained from a trial adsorption isotherm experiment: 120, 150, 180, 210, and 240 mg kg^{-1} for Zn and Cd and 150, 200, 250, 300, 350, and 400 mg kg^{-1} for Pb. The centrifuge tubes were shaken at room temperature for 24 hours on an end-over-end shaker at 20 rpm. A spike (0.40 mL) of enriched stable isotopes was then added from stock solutions with concentrations of 30.8, 38.3 and 54.2 mg L^{-1} of ^{70}Zn, ^{108}Cd and ^{204}Pb, respectively and the resin suspensions were shaken again for 24 hours. The supernatant was separated by centrifuging for 15 min at 2500 rpm followed by filtration (0.22 µm, Millex® Millipore). The liquid phase was acidified to a final concentration of 2% HNO_3 prior to assay of isotope ratios by ICP-MS.

2.2.2 Measuring isotopically exchangeable metal (E-values) in soil

Soil samples (c. 1.0 g) were suspended in 30 mL of 0.01 M $Ca(NO_3)_2$ and shaken end-over-end in Oak Ridge polypropylene centrifuge tubes for 3 days. The suspensions were then spiked with a small volume of solution containing enriched ^{70}Zn, ^{108}Cd and ^{204}Pb. A range of spike concentrations were added to the soil suspensions according to the anticipated labile pool of ^{70}Zn, ^{108}Cd and ^{204}Pb content in each soil. It is recognised that the spike added to the soil must significantly alter the natural abundance of the spiked isotope to be accurately measured by ICP-MS (Ayoub et al., 2003, Gabler et al., 2007, Gabler et al., 1999). Therefore, the labile pool of these metals in each soil was initially estimated by conventional DTPA extraction (described in Section 2.3). The aim was to determine a spike concentration able to produce a quantifiable change in isotope ratio, without affecting the apparent E-value, and at minimal experimental cost.

An initial ID experiment was undertaken using multi-element spike concentrations equivalent to a 50 % change in the natural abundance of the labile pool of ^{70}Zn, ^{108}Cd and ^{204}Pb content in the soil based on the amount of Zn, Cd and Pb extracted by DTPA. According to the E-values obtained from this experiment, 5 levels of multi-element spike solution were then applied to soil suspensions equivalent to, approximately, 10, 30, 50, 70 and 100 % change in the natural abundance of the labile pool of the three spike isotopes (^{70}Zn, ^{108}Cd and ^{204}Pb). The soil samples were divided into three groups, according to the E-value estimated from the initial ID experiment, and variable amounts of the working spike solution, prepared from the acidified spike stock solution, were added to triplicate soil suspensions as aliquot volumes: 0.1, 0.3, 0.5, 0.7 and 1.0 mL. The concentrations of ^{70}Zn, ^{108}Cd and ^{204}Pb isotopes in the working spike solution were 25.1, 0.712 and 29.3 mg L^{-1} in group one, 4.11, 0.0805 and 18.1 mg L^{-1} in group two and 0.389, 0.0383 and 30.9 mg L^{-1} in group three

respectively. The effect on suspension pH of adding variable amounts of the working spike solution was determined for the highest and lowest spike levels. The different spike concentrations were added to the soils collected from Derbyshire, UK (see chapter 3) in terms of selecting the promising spike concentration and then only one concentration have been applied for the other experiments as stated in each chapter. The spiked and un-spiked soil suspensions were re-equilibrated for a further 3 days. The supernatant was then separated as described in section 2.2.1 and used to measure isotope ratios for Zn, Cd and Pb by ICP-MS.

A further step to improve the determination of E-value, suggested by Hamon and McLaughlin (2002) and Lombi et al. (2003), was introduced to determine the existence of metals present in the solution phase as non-exchangeable forms in colloids < 0.20 µm. Interference from 'non-labile colloidal' (NLC) metal was assessed using equilibration of filtered soil solutions with Chelex-100 resin. The underlying principle is that a cation exchange resin will adsorb only labile metal, which has equilibrated with the added isotopic spike, while the non-labile metal, supposedly held in sub-micron particulate form, remains suspended in the solution phase. Analytical grade Chelex-100 (Bio-Rad laboratories, UK) was supplied in sodium form as wet beads (150-300 µm). The Na-Chelex-100 was converted into the Ca form by allowing it to equilibrate with 0.5 M $Ca(NO_3)_2$ at 25 °C for 2 hours and then centrifuging for 15 min at 2500 rpm. The resin was then washed twice with MilliQ water to remove residual salt. Conversion to the Ca-resin reduced the pH of the Chelex-100 from 10.05 to pH 7.90. An aliquot of approximately 100 mg of Ca-Chelex-100 resin was shaken with 10 mL of the filtered supernatant, from spiked and un-spiked soil suspensions, for 2 hours. After washing the resin several times with MilliQ water, an elution step was undertaken with 0.5 M HNO_3 for 30 min. The resulting E-value (E_{resin}) was determined, based on isotope ratios in the resin phase, and compared to the

conventional value based on isotope ratios in the solution phase (E_{soln}). It is then possible to quantitatively express the amount of metal (mg kg^{-1} soil) held in *suspended* NLC simply as the difference between E_{resin} and E_{soln}.

2.2.3 Calculating E-value

The concentrations of isotopically-exchangeable trace metals (E-values) were determined from the ratio of the main 'spike isotope' to one of the major background isotopes. The key measurement is the ratio of isotopic abundances of a light and heavy isotope in the un-spiked soil solution ('soil') and in the spiked soil solution ('spike+soil'). For the three trace metals assayed, the ratios of isotopic abundances used were (^{70}Zn:^{66}Zn), (^{108}Cd:^{111}Cd) and (^{204}Pb:^{208}Pb). Therefore, E-value is calculated from equation 2.1.

$$E-value= \left(\frac{Am_{M_{soil}}}{W}\right)\left(\frac{C_{spike}V_{spike}}{Am_{M_{spike}}}\right)\frac{(^{lighter}IA_{spike}-{}^{heavier}IA_{spike}R_{ss})}{(^{heavier}IA_{soil}R_{ss}-{}^{lighter}IA_{soil})} \qquad (2.1)$$

W is the weight of soil used (kg), V_{spike} is the volume of spike added (L), IA is isotopic abundance (mole basis), Am_M is the average atomic mass of the metal in the spike or the soil, C_{spike} is the gravimetric concentration of the metal in the spike solution and R_{ss} is the ratio of isotopic abundances (light:heavy) for the two isotopes in the spiked soil solution.

2.2.4 Experimental and analytical conditions

Initially, unexpectedly large signals for ^{70}Zn (0.6 % natural abundance) were recorded in blank solutions when running ICP-MS in standard mode. This was thought to be due to interference from a chlorine dimer (^{35}Cl-^{35}Cl) (Stenberg et al., 2004, Malinovsky et al., 2005) and was resolved by operating in 'collision cell with kinetic energy discrimination' mode (CCT-KED). All extracted solutions were diluted to fall in the concentration range 5 and 50 µg L^{-1} for Zn, Cd and Pb. This

was essential for isotope ratio measurements to prevent the ICP-MS detector from switching to 'analogue' measurement mode in response to high (typically > 100 μg L^{-1}) concentrations which produced count rates in excess of (typically) 1.50 million cps. It was found that isotope ratios composed of mixed 'analogue' and 'pulse counted' measurements (eg ^{208}Pb and ^{204}Pb) were reasonably reliable but subject to greater error than pulse-counted measurements even if an *internal* cross-calibration factor was applied. A range of standard concentrations (0 – 30 μg L^{-1}) was used to optimize the instrumental dead time correction factor by resolving the setting that eliminated isotopic ratio dependence on analyte concentration (see Fig. 2.1 for calculating the detector dead time correction using Pb SRM NIST 981).

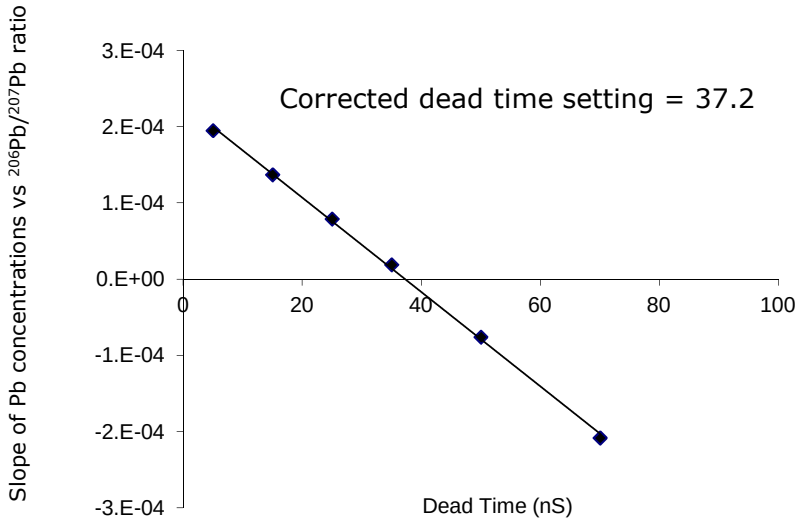

Fig. 2.1 Calculation of detector dead time using Pb SRM NIST 981.

Mass discrimination problems were resolved through a correction factor (the 'K factor') which is simply the ratio of 'true' to 'measured' isotopic ratios (IR_t and IR_m respectively, equation 2.2), determined from isotope reference standards.

$$\text{K-Factor} = \frac{\boldsymbol{IR_t}}{\boldsymbol{IR_m}} \qquad (\mathbf{2.2})$$

Values of K-factor were determined, at periodic intervals during a sample run, using the standard reference material NIST SRM-981 (lead wire) for Pb and normal ICP-MS calibration standards for Zn and Cd, all at 25 μg L^{-1}. The certified isotopic reference was required for Pb because of the variability of Pb isotope ratios arising from different radiogenic origins (Komarek et al., 2008). It is expected that K-factors will decrease with mass, for a given isotopic mass difference, as discrimination effects are greater at lighter masses (Xie and Kerrich, 2002, Nolan et al., 2004). However, in early trials, the K-factor for ^{64}Zn:^{66}Zn was found to be erroneously large. This may have occurred because the quadrupole did not have sufficient time to stabilize before measuring the 'first' (lightest) isotope at the low dwell times (2.5 ms) used to measure isotope ratios to minimise plasma flicker. The problem was resolved by adding one isotope (^{63}Cu) to the measurement sequence, immediately below ^{64}Zn, to give the quadrupole sufficient time to 'locally stabilise'. The isotopes ^{107}Ag and ^{202}Hg were also measured just below the lightest Cd and Pb isotopes for the same reasons; ^{202}Hg was also used to correct for ^{204}Hg interference with ^{204}Pb (Walder et al., 1993).

To minimise the error caused by ICP-MS plasma noise interferences, it may be worth measuring as few isotopes as possible, and inferring the others according to their natural abundance. According to initial trial results, the two major isotopes for Cd and Zn were determined and the other isotopes were inferred according to their natural abundance. For example, Cd has 8 isotopes and the

variation in plasma noise during measurement is likely to affect the accuracy of measurements while scanning from the first isotope to the last one. Therefore, there is a trade off between measuring all isotopes and risking problems with plasma noise or inferring the isotopic abundance of several isotopes and risking the errors this will entail. Precision is often inadequate for isotope-ratio determinations, especially when too many isotopes must be examined in a relatively short time (Hieftje et al., 2001). This approach is not possible for Pb, for which all (four) isotopes must be measured, as each soil will have a unique isotopic signature (Komarek et al., 2008). The sequence of isotope analysis and quadrupole dwell times used in the spiked and unspiked samples were as follows: ^{63}Cu (5.0 ms), ^{66}Zn (2.5 ms), ^{70}Zn (10 ms), ^{107}Ag (5.0 ms), ^{108}Cd (10 ms), ^{111}Cd (2.5 ms), ^{202}Hg (10 ms), ^{204}Pb (10 ms), ^{206}Pb (2.5 ms), ^{207}Pb (2.5 ms) and ^{208}Pb (2.5 ms). The operating conditions for the ICP-MS analysis are summarized in Table 2.2 for determination of isotopic ratio and multi element analysis.

2.3 Single and sequential extraction procedures

Triplicate samples of 4.0, 2.0 and 2.0 g soil (< 2 mm sieved) were suspended in 8 mL of 0.005 M DTPA (Lindsay and Norvell, 1978), 10 mL 0.05 Na_2-EDTA (Quevauviller et al., 1996) and 10 mL of 0.43 M HNO_3 (Groenenberg et al., 2010), respectively and shaken on an end-over-end shaker, in Oak Ridge polypropylene centrifuge tubes, for 2 hours. Suspensions were then centrifuged at 2500 rpm for 15 min and the supernatant filtered (<0.2 μm) into polyethylene tubes containing sufficient HNO_3 to produce 2% acid prior to multi-element analysis by ICP-MS, as described in Section 2.3.

Table 2.2 ICP-MS operating conditions for both multi element and isotope ratio analysis in CCT-KED mode

Parameter	**Value and/or description**	
	Isotope ratio analysis	**Multi element analysis**
Forward power (Watts)	1404	1404
Reflected power (Watts)	< 5.0	< 5.0
Nebulizer gas flow rate ($Lmin^{-1}$)	0.84	0.84
Lens 1 (V)	-1160	-1160
Lens 2 (V)	-69.0	-69.0
Focus (V)	-6.1	-6.1
D1 (V)	-46.3	-46.3
D2 (V)	-124	-124
Hexapole bias (V)	-18.0	-18.0
Pole bias	-14.0	-14.0
Reaction cell gas	4 mL min^{-1} of 7% H_2 in He	4 mL min^{-1} of 7% H_2 in He
Detector dead time nS	35.0	55.0
Quadruple parameters		
Maximum settle time (μS)	1000	15000
Minimum settle time (μS)	1000	1500
Safe resting mass (amu)	205	150

Sequential chemical extraction was carried out following the method described by Li and Thornton (2001), developed from that of Tessier et al. (1979). The extraction was carried out progressively on an initial soil weight of 1.0 g. The sequence of extraction was as follows:

1. Fraction 1 – 'Exchangeable': 1 g soil was extracted for 20 min with 8 ml 0.5 M $MgCl_2$.
2. Fraction 2 – 'Carbonate bound' and 'specifically adsorbed': residual soil from step 1 was extracted with 8 ml 1 M NaOAc (adjusted to pH 5 with HOAc) for 5 hours.

3. Fraction 3 – 'Bound to Fe/Mn oxides': the residue from step 2 was extracted with 20 ml 0.04 M NH_2.OH.HCl in 25% (v/v) HOAc at 96 ^oC in a water bath for 6 h with occasional agitation.

4. Fraction 4 – 'Bound to organic matter and sulfides': the residue from step 3 was extracted with 3 ml 0.02 M HNO_3 and 5 ml 30% H_2O_2 (adjusted to pH 2 with HNO_3). Samples were heated to 85 ^OC in a water bath and maintained for 2 hours with occasional agitation. Three ml H_2O_2 were then added and the mixture was heated again for 3 hours at 85 ^OC with intermittent agitation. After cooling, 5 ml 3.2 M NH_4OAc in 20% HNO_3 (v/v) was added. The tubes were then agitated for 30 min.

5. Fraction 5 – 'Residual': the residue from step 4 was digested with HNO_3, HF and $HClO_4$ as described in section 2.1.2.

All extractions, except the last one, were undertaken in 50 ml Oak Ridge polypropylene centrifuge tubes. After each successive extraction, the supernatant solution was separated by centrifugation and filtration as described in sections 2.2.1 and 2.2.2. The final extraction step (F5) was carried out following the procedures described in section 2.1.2. The concentration of metals in all the extracted solutions was measured by ICP-MS.

The recovery of the sum of fractions for all analyzed elements was compared with elemental concentrations from the single acid digestion procedure (section 2.1.2). The SEP was also applied to a reference soil material (Montana soil SRM 2710) and the recovery of the sum of the fractions was compared with the accredited total metal content. However, no certified reference materials were available for the individual sequential extraction steps, probably because there is insufficient agreement on standard methods of extraction (Li et al., 1995).

2.4 Geochemical modelling of metal solubility by WHAM-VI

To predict metal solubility and fractionation in the whole soil-solution system, a geochemical speciation software package was employed: The extended version of WHAM/Model VI (Windermere Humic Aqueous Model; hereafter WHAM(VI)) which includes consideration of bonding to metal oxides and alumino-silicate clays (Tipping et al., 2003). Speciation was undertaken for data derived from the suspensions used to measure E-values: soil was suspended in either 0.01 M $Ca(NO_3)_2$ (section 4.2.3) or 0.001 M $CaCl_2$ (section 5.2.5) at a liquid:soil ratio of 30:1 or 30:5 respectively, and the model was assessed in terms of its ability to predict overall metal solubility in the liquid phase of the suspensions.

Default parameter settings and input files were used for WHAM(VI). The model simulation were used to predict the total metal concentration in the solution phase in the suspensions of the studied soils (n = 246), using the following input parameters: either E-value or *total* metal concentration of Zn, Cd and Pb (mol L^{-1}), the soil pH, the particulate phases (organic matter, Fe- & Mn-oxides concentration, g L^{-1}), solution concentrations of [Al], [Fe^{+2}] [Na], [Mg], [K], [Ca], [NO_3] (mol L^{-1}) as dissolved species and a fulvic acid colloidal phase (from dissolved organic carbon, DOC); the temperature was set to 25°C. The DOC concentration was converted to colloidal fulvic acid by assuming that DOC contains 50% C and that 65% of the DOC measured in 0.01 M $Ca(NO_3)_2$ solution was 'active' FA, the remainder being inert with respect to ion binding (Cheng et al., 2005, Buekers et al., 2008a). Soil organic matter (as % LOI) was converted to humic acid by assuming that 50% of this organic matter consists of active Humic material (Buekers et al., 2008b). The partial CO_2 pressure was set to the atmospheric value ($pCO_2 = 3.83\times10^{-4}$). Changing pCO_2 from 1 to 10 times atmospheric level had a minimal effect on Zn, Cd, or Pb speciation in the soil pore water (Nolan et al., 2003). DOC, Fe-oxide (FeOOH) and Mn-oxide (MnO_2) content were determined as described in section 2.1.2. E-values and total metal

concentrations were determined according to the methods described in sections 4.2.3 and 2.1.2 respectively. The total suspended particulate material SPM (g L^{-1}) was calculated according to the soil:liquid ratio used in the suspensions used to determine isotopically exchangeable metal (e.g. 1g of soil to 30 mL $Ca(NO_3)_2$).

Output from WHAM(VI) includes the total soluble metal concentration (mol L^{-1}) and the proportion of different fractions associated with soil particulate and colloidal phases.

2.5 Statistical analysis

Analysis of variance (ANOVA), paired T-tests and Pearson correlation coefficients were determined using Minitab ® 15.1.3.0.; the default level of confidence was 95.0% ($P < 0.05$) unless stated otherwise in the discussion.

3. CHAPTER THREE: USING MULTI-ELEMENT STABLE ISOTOPE DILUTION TO QUANTIFY METAL REACTIVITY IN CONTAMINATED SOILS

3.1 OVERVIEW

Soil is a complex system compared to single natural, or synthetic, absorbents. Trace metals in soil may be associated with many different mineral and organic phases or exist as discrete compounds and complexes in the soil solution phase. The proportion of the various metal pools depends mainly on (i) transient soil properties (pH, Eh, temperature etc), (ii) soil constituents (humus, Fe/Mn oxides, clay minerals, carbonates and phosphates), (iii) the original form of the metal contamination and (iv) contact time (Daviescolley et al., 1984, Ledin et al., 1996, Degryse et al., 2004, Hammer et al., 2006, Tack, 2010). Therefore the total concentration of metals in such complex systems does not provide sufficient insight into their lability, solubility and bioavailability (He et al., 2005). Hence, measurement of metal lability is important for the evaluation of metal toxicity in contaminated soils (Midwood, 2007). The development of multi-element stable isotope dilution is described in Chapter 2 for simultaneous determination of the isotopically exchangeable pools of Zn, Cd and Pb in contaminated soils from old Pb/Zn mining areas in Derbyshire, UK. There is a pressing need to establish the simplicity and the cost-effectiveness of this technique so that it might ultimately be used routinely as current extraction procedures, possibly employing commercially available liquid isotopic spike solutions.

The immediate aim of this Chapter is therefore to develop a reliable multi-element stable isotope dilution technique and compare this with single and sequential extractions to assess the chemically reactive pools of Zn, Cd and Pb in a wide range of contaminated soils.

3.2 MATERIALS AND METHODS

Most of the analytical methods used in this chapter are described in details in Chapter 2. The following information describes the study area and some additional methods not previously described.

3.2.1 Study area and soil samples

Soil samples were taken from two sites in Derbyshire (UK) within the old Pb/Zn mining areas of Darley Dale (Clough Wood; 53°09'08" N, 1°36'57" W, Elev. 148 m) and Matlock (Black Rocks; 53°05'53" N, 1°33'52" W, Elev. 250 m). The study area is in a valley trending north-west to south-east, which is located approximately 27 km to the south-west of Sheffield (Fig. 3.1 and 3.2). Lead and zinc have been mined in the region since the 17th century and production peaked between 1859 and 1939 (Young et al., 2000 and refrences cited thierin). The mine in Clough Wood, Millclose, was the deepest mine in Derbyshire (>1000 feet). The surface geology at Clough Wood is carboniferous shale while at the Black Rocks site it is Gritstone. Both these parent materials give rise to acidic soils and this is accentuated by the presence of mixed woodland at both sites. However, the underlying formation is Carboniferous limestone and the Pb/Zn mineralogy has formed at the interface of the limestone and overlying formations. Therefore, mining, ore processing and smelting activities have led to surface deposition of both heavy metal contaminated materials and accompanying limestone. This has given rise to a wide range of soil conditions as well as highly variable levels of metal contaminant loadings. The soil samples collected were intended to represent a wide range of soil characteristics likely to affect metal reactivity: pH, %C and metal concentrations. Eight soil samples (0-15 cm) were taken from both sites. Six samples were taken from Clough Wood: three from woodland; CW-W1, CW-W2 and CW-W3, two from minespoil area; CW-MS1 and CW-MS2 and one from grassland; CW-G. From the Black Rocks site, two topsoil samples (0-15cm) were taken: one from woodland, BR-W and

one from the minespoil area, BR-MS (Plates 3.1, 3.2 and 3.3). All soil samples were air-dried, sieved and prepared for chemical analysis and isotopic exchange experiments as described in sections 2.1.2 and 2.2.2.

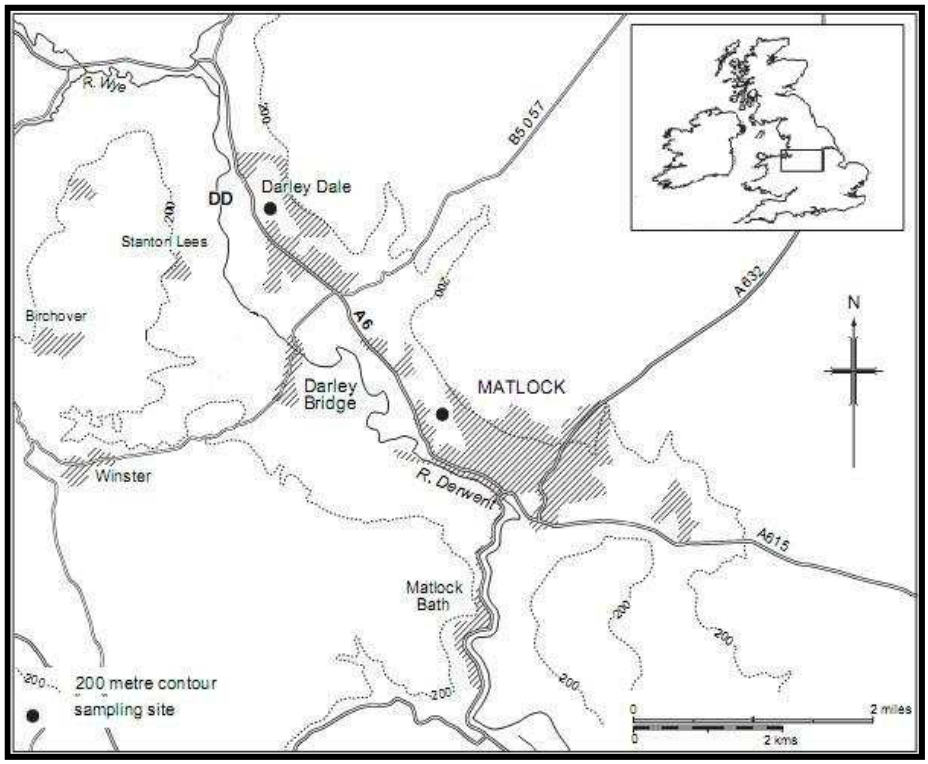

Fig. 3.1 Sampling locations near Darley Dale (Clough Wood) and Matlock (Black Rocks). (Adapted from Lageard et al., 2008).

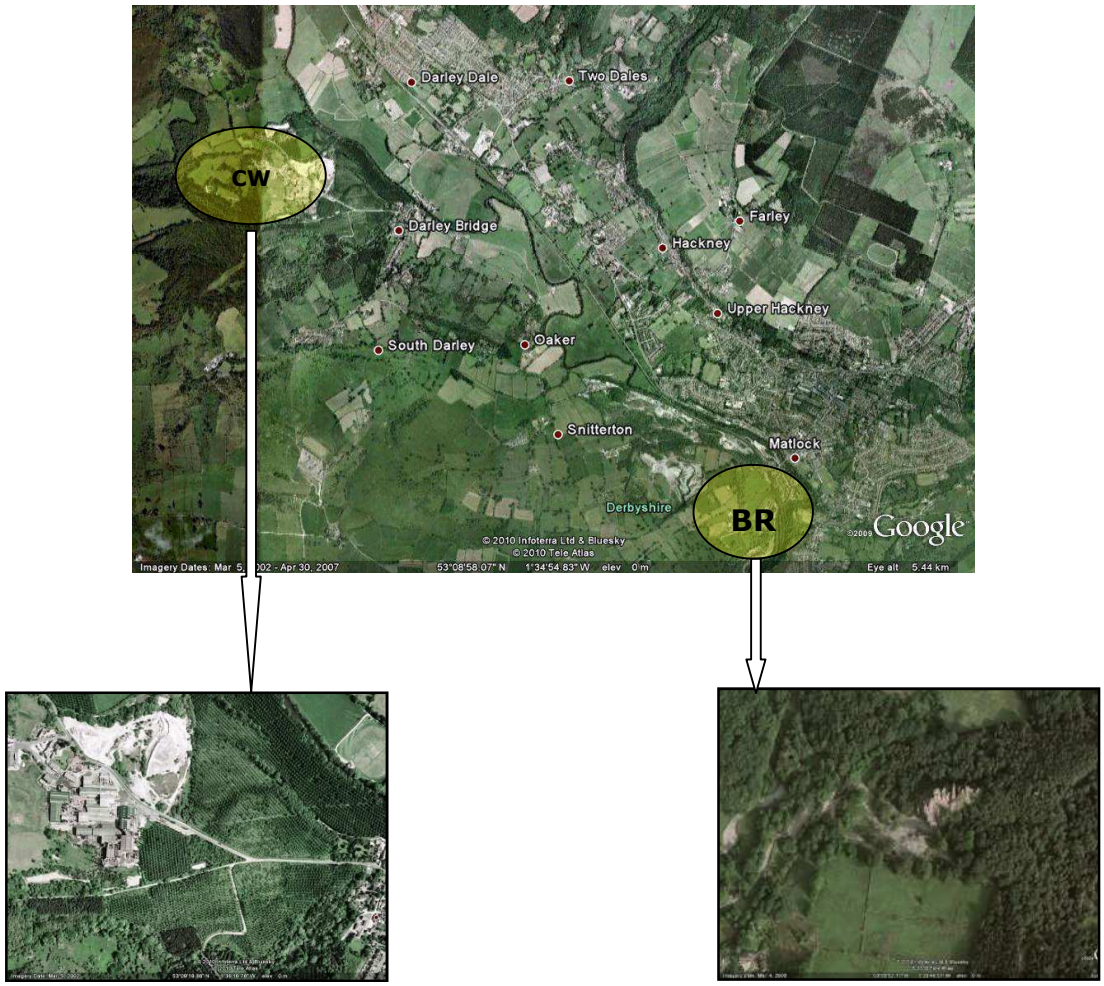

Fig. 3.2 A satellite labelled view of Clough Wood (CW) and Black Rocks (BR) sites. *Source*: ''Darley Dale.'' 53° 08' 58.07'' N and 1° 34' 54.83'' W. **Google Earth**. Updated and composed on 5th March 2002 – 30th April 2007. Accessed on 8th February 2010.

Plate 3.1 Clough Wood site photographed from the main minespoil area over the original mine-shaft showing various mounds of spoil material and resorted waste from ore separation activities. The valley bottom has a partially culverted stream and Cambridge Wood is shown on the other side of the valley.

Plate 3.2 Clough Wood site showing the steep minespoil deposit leading up to the top of the original mineshaft and the remnants of the 'Watts engine house'.

Plate 3.3 The minespoil area of the Black Rocks site showing calcareous minespoil scree leading up to the mineshaft at the base of the Carboniferous gritstone outcrop.

3.3 RESULTS AND DISCUSSION

3.3.1 General soil characteristics

The vegetation at the site of the eight soils and their main chemical and physical soil characteristics are shown in Tables 3.1 and 3.2. Relatively high pH values were found in the three soils developed directly on minespoil: BR-MS, CW-MS2 and CW-MS1 had pH values of 7.42, 7.29 and 7.05, respectively and the corresponding soil carbonate contents were 31.5, 2.01 and 3.37%. Soil pH values were negatively correlated with LOI ($r = -0.45$) reflecting a contrast between soils directly affected by calcareous minespoil and the woodland soils from the Clough Wood site, developed on Carboniferous shale, which were the most acidic; soils CW-W2, CW-W3 and CW-W1 had pH values of 3.46, 4.30 and 5.13, respectively. Intermediate pH values of 5.72 and 6.36 were found in soils CW-G and BR-W, respectively. Although soil BR-W had the highest LOI (56.1%), the pH was close to neutral (pH 6.36) due to small amounts of carbonate

material covering the site from a steep slope extending from the original mine shaft.

Table 3.2 shows total metal concentrations determined for the eight soil samples. The acid digestions of the soil samples and subsequent analysis using ICP-MS were performed twice to check analytical and procedural precision. The results showed less than 1% variability (eg. Zn, Cd and Pb, Fig. 3.3), confirming the overall reproducibility of the digestion protocol and analysis by ICPMS.

Soil metal concentrations ranged from 112-43,300 mg Zn kg^{-1}, 2.53-668 mg Cd kg^{-1}, and 552-30,000 mg Pb kg^{-1}, reflecting the degree of historical exposure to smelter fallout and minespoil. Such high concentrations of Pb and Zn have been reported in soils from many mining areas of the world, such as Derbyshire (Li and Thornton, 2001) and Bukowno in southern Poland (Verner et al., 1996). Soil Zn, Pb and Cd concentrations are frequently correlated because these ore body metals are often found together, associated with sulphur, in primary minerals (Li and Thornton, 2001). There was a highly significant correlation between Pb and both Cd ($r = 0.97$; $P<0.001$) and Zn ($r = 0.97$; $P<0.001$) over all the sampling points (Table 3.3). A stronger correlation was also found between Zn and Cd concentrations (in mg kg^{-1}) (*Zn = 66.6 Cd - 31.9*; $r = 0.999$; $P<0.001$) where the slope of the linear equation corresponds to a Zn:Cd atom ratio of 114:1 (Fig. 3.4). This is presumably due to the fact that Cd is directly isomorphously substituted for Zn in the sulphide ore mineral sphalerite (Nakhone and Young, 1993).

Table 3.1 Soil vegetation cover, pH, total carbonate concentration, loss on ignition (LOI) and available and total Phosphorus concentrations. Data are arranged in descending order of pH value.

Soil Samples*	Vegetation cover	pH	Carbonate (%)	%LOI	Phosphorus (mg kg^{-1})	
					Available P	Total P
BR-MS	Spoil heap	7.42	31.5	4.31	8.38	582
CW-MS2	Spoil heap	7.29	2.07	5.33	3.63	669
CW-MS1	Spoil heap	7.05	3.37	8.74	3.8	604
BR-W	Spoil heap	6.36	0.42	56.1	40.3	1260
CW-G	Grassland	5.72	0.16	19.3	9.93	1760
CW-W1	Woodland	5.13	0.07	30.1	4.75	664
CW-W3	Woodland	4.30	1.00	11.0	1.78	769
CW-W2	Woodland	3.46	n.d	41.9	2.93	845

*(BR) Black Rocks; (CW) Clough Wood; (MS) Minespoil; (W) Woodland; (G) Grassland

Table 3.2 Element concentrations in soils from Clough Wood and Black Rocks. Standard deviations are shown in brackets.

Soil samples	Mg	Ca	Mn	Fe	V	Cr	Co	Ni	Cu	Zn	Cd	Ba	Pb	U
	(g kg^{-1})				(mg kg^{-1})									
CW-MS1	2.71	169	553	32.3	88.3	50.3	12.0	95.9	143	27600	412	4490	29300	5.66
	(0.05)	(2.10)	(12.7)	(0.53)	(1.35)	(0.25)	(0.16)	(4.86)	(4.26)	(443)	(9.77)	(1549)	(1430)	(0.02)
BR-MS	2.00	169	661	13.0	48.4	27.6	7.57	32.2	228	43300	669	2220	23700	4.29
	(0.02)	(2.15)	(13.0)	(0.10)	(0.94)	(0.54)	(0.29)	(1.00)	(8.70)	(1205)	(24.8)	(479)	(392)	(0.09)
CW-MS2	2.42	146	403	31.8	64.6	46	13.3	70.0	140	39800	580	2570	29900	4.47
	(0.04)	(0.89)	(1.87)	(0.17)	(1.17)	(0.33)	(0.28)	(5.12)	(2.42)	(928)	(22.2)	(427)	(113)	(0.13)
CW-G	3.61	57.1	1227	31.7	127	69.6	20.8	73.9	100	3470	51.3	15300	10300	8.39
	(0.08)	(1.43)	(24.5)	(0.02)	(1.99)	(1.01)	(0.50)	(1.88)	(4.15)	(333)	(5.49)	(501)	(364)	(0.13)
BR-W	1.09	23.3	1128	7.13	20.4	14.2	3.43	17.4	36.1	1260	19.1	3440	1300	0.79
	(0.02)	(0.52)	(11.6)	(0.15)	(0.24)	(1.48)	(0.09)	(12.6)	(2.30)	(317)	(5.81)	(240)	(8.77)	(0.01)
CW-W1	4.31	3.61	693	30.2	169	87.7	13.7	16.5	55.3	143	3.66	444	667	6.76
	(0.09)	(0.10)	(21.6)	(0.07)	(2.71)	(1.18)	(0.11)	(0.87)	(0.70)	(43.0)	(0.42)	(48.3)	(7.0)7	(0.15)
CW-W2	3.03	2.99	116	22.3	130	70.4	4.42	22.3	119	112	5.70	650	2450	5.46
	(0.04)	(0.03)	(1.77)	(0.03)	(0.27)	(0.21)	(0.09)	(7.55)	(3.93)	(7.70)	(0.25)	(11.6)	(5.65)	(0.03)
CW-W3	4.85	2.76	239	37.1	206	110	6.86	19.6	48.0	121	2.54	574	553	9.23
	(0.03)	(0.09)	(2.87)	(0.05)	(0.70)	(1.01)	(0.24)	(0.09)	(0.65)	(16.4)	(0.33)	(12.7)	(11.0)	(0.12)

Table 3.3 Correlation matrix for the measured elemental analysis of Clough Wood and Black Rocks soils.

	Mg	Ca	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Ba
Ca	-0.457											
	*0.255**											
V	**0.982**	-0.575										
	0.000	*0.136*										
Cr	**0.984**	-0.541	**0.994**									
	0.000	*0.166*	*0.000*									
Mn	-0.335	0.018	-0.397	-0.431								
	0.418	*0.967*	*0.330*	*0.287*								
Fe	**0.805**	-0.032	**0.734**	**0.786**	-0.355							
	0.016	*0.940*	*0.038*	*0.021*	*0.388*							
Co	0.359	0.230	0.211	0.240	0.412	0.584						
	0.383	*0.584*	*0.616*	*0.567*	*0.310*	*0.129*						
Ni	-0.111	0.695	-0.222	-0.170	0.154	0.428	0.640					
	0.793	*0.056*	*0.598*	*0.687*	*0.716*	*0.290*	*0.087*					
Cu	-0.353	**0.821**	-0.425	-0.415	-0.181	-0.163	0.080	0.399				
	0.392	*0.012*	*0.294*	*0.306*	*0.667*	*0.700*	*0.850*	*0.328*				
Zn	-0.449	**0.947**	-0.562	-0.519	-0.132	-0.088	0.102	0.492	**0.848**			
	0.264	*0.000*	*0.147*	*0.187*	*0.755*	*0.835*	*0.810*	*0.215*	*0.008*			
Cd	-0.451	**0.946**	-0.562	-0.523	-0.130	-0.105	0.090	0.478	**0.86**	**0.999**		
	0.262	*0.000*	*0.147*	*0.184*	*0.760*	*0.805*	*0.831*	*0.231*	*0.006*	*0.000*		
Ba	-0.036	0.168	-0.121	-0.109	0.687	0.165	**0.748**	0.605	0.051	-0.024	-0.030	
	0.933	*0.691*	*0.775*	*0.798*	*0.060*	*0.696*	*0.033*	*0.112*	*0.904*	*0.954*	*0.943*	
Pb	-0.414	**0.991**	-0.533	-0.492	-0.062	0.014	0.244	0.682	**0.852**	**0.968**	**0.965**	0.145
	0.308	*0.000*	*0.173*	*0.216*	*0.883*	*0.973*	*0.560*	*0.062*	*0.007*	*0.000*	*0.000*	*0.732*

*Italic and bold figures represent P and significant values, respectively.

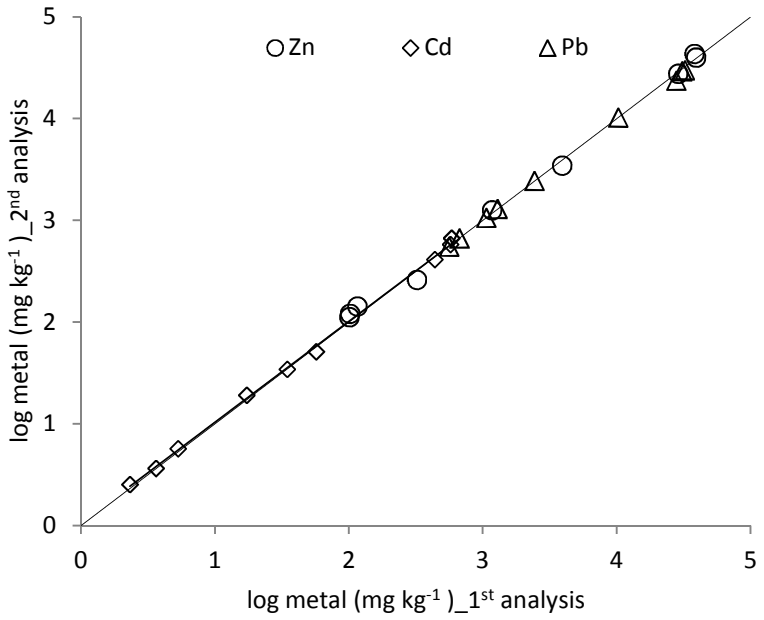

Fig. 3.3 Correlation of Zn (○), Cd (◇) and Pb (△) (log values) for the 1st and 2nd metal digestion and analysis procedures undertaken using the same analytical methods (section 2.1.2) as a test of methodological precision; solid line represent the 1:1 relation.

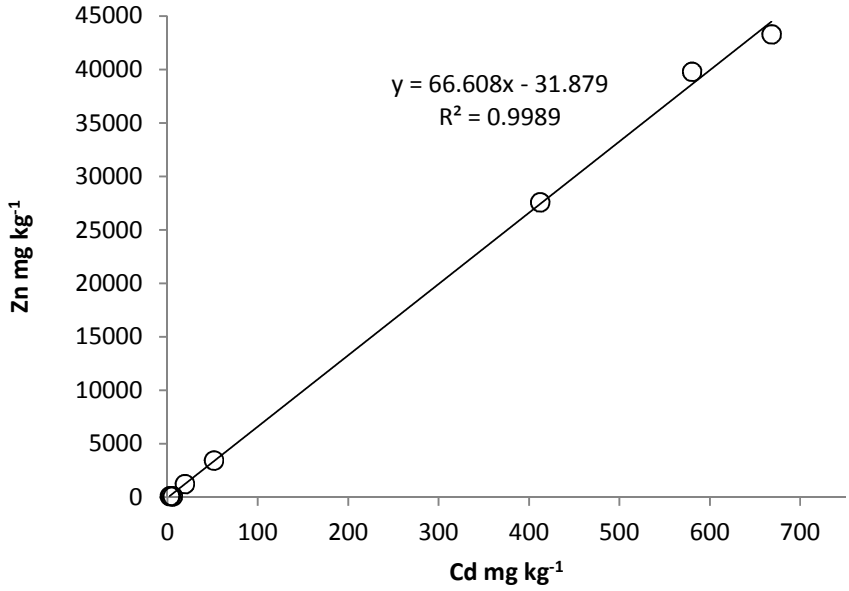

Fig. 3.4 Relationship between the concentration of Cd and Zn (mg kg^{-1}) in the soils from Black Rocks and Clough Wood, Derbyshire, UK.

3.3.2 Preliminary test of ID methodology

Recovery experiments with Zn, Cd and Pb added to resin-electrolyte (Amberlite IR 120 in 0.01M $Ca(NO_3)_2$) suspensions were performed to determine whether added metals were fully accessible to isotopic dilution. If the added metal ions remain isotopically exchangeable and do not irreversibly bond to the resin, then the isotopic spike must fully mix with the sorbed metal pool and the added metal will be determined to be 100% labile. Following several trials to optimize the instrumental and experimental conditions, Zn, Cd and Pb showed average % lability of 100, 97.8 and 98.7 with standard errors of 2.20, 0.71 and 1.60 respectively. This confirmed the validity of the ID technique used with a mixed spike solution under different metal concentrations. Many researchers have verified that ID techniques used to determine isotopically exchangeable pools in soil are consistent over a wide range of experimental conditions (Gabler et al., 1999, Young et al., 2000, Gabler et al., 2007, Sterckeman et al., 2009, Atkinson et al., 2011).

3.3.3 E-value

3.3.3.1 Effect of spike concentration and pH on E-value

The amount of stable isotope spike added to the soil suspension should not affect the measured E-value although it may change the equilibrium between labile metal in the solid and solution phases (Sterckeman et al., 2009). Nevertheless, there was some concern that the acidic spike solution might change the soil suspension pH and, thereby, the lability of the soil metals. Gabler et al. (2007) and Sterckeman et al. (2009) reported that changes in metal lability may arise if the added spike solution contains sufficient acid to change conditions in the equilibrating suspension. Sterckeman et al. (2009) recommended using a spike solution with pH ranging from 4.5 to 5.5 to guarantee the stability of measured E-values. However, in the current study, pH

values measured in different soil suspensions at the maximum and minimum spike loadings were not significantly different (P = 0.24) (Table 3.4).

Table 3.4 Effect of different amount of the spike solution on the soil suspension pH.

Soil Samples	**pH in the spiked soil after equilibration time**	
	Lowest spike solution (0.1 mL)	Highest spike solution (1.0 mL)
BR-W	5.88	5.88
CW-W1	4.84	4.83
CW-W2	3.95	3.93
CW-W3	3.11	3.11
CW-MS1	6.74	6.74
CW-MS2	7.09	7.02
CW-G	5.59	5.48
BR-MS	7.18	7.22
Average	5.55	5.53

Figure 3.5 shows the effect of increasing ^{70}Zn, ^{108}Cd and ^{204}Pb spike on the determination of the 'apparent' E-values for Zn, Cd and Pb in Clough Wood and Black Rocks soils from isotope ratios measured either in the solution (E_{soln}) or Chelex-100 resin phases (E_{resin}). In general, good agreement was found for different spike concentrations across a wide range of soil characteristics. The results confirm that even a small amount of the spike isotope added to the soil, equivalent to approximately 10 % of the native labile metal pool of ^{70}Zn, ^{108}Cd and ^{204}Pb isotopes in the soil, will provide sufficient change in isotopic abundance to be reliably measured by ICP-MS. The results for the current work suggest that adding the spike isotope according to the native labile isotope estimated by 0.05 M DTPA leads to an analytically reliable change in the isotopic natural abundance. Ayoub et al (2003) suggested that the spike isotope would create a measurable change in Cd and Zn isotopic ratios by altering the $^{114}Cd/^{111}Cd$ ratio

from just 2.22 to 1.96, and the $^{66}Zn/^{67}Zn$ ratio from 6.76 to 6.10. The amount of Cd and Zn spike added to the soil to achieve these isotopic ratio changes was based on the amount of Zn and Cd extracted by 0.43 M CH_3COOH.

In general, E-values appeared consistent against different spike levels with little bias (Fig. 3.5). Nevertheless, the spike levels added to the soil suspension showed a small statistically significant effect on measured E-value in all soils ($P \leq 0.03$) except CW-W1 and CW-W2 ($P \geq 0.08$) for Pb, in BR-W and CW-W1 for Cd ($P \leq 0.04$) and in CW-G for Zn ($P < 0.05$). The effect of spike level on apparent E-value was greatest in soils with high organic matter and carbonate contents. For instance, in the case of BR-W and BR-MS which contained the highest organic matter content (56.1 % LOI) and the highest carbonate content (31.5 %) respectively, the different amounts of spike added had a highly significant effect on apparent E-value for Pb in both soils ($P \leq 0.001$) and for Cd in BR-W ($P < 0.0001$). However, the changes in Zn, Cd and Pb E-value were not consistent and might be partly attributed to colloidal interferences from dissolved or suspended humic acid in the woodland soils suspension. This appears particularly plausible for Pb in the soils with higher humus content and pH value because when a Chelex-100 resin purification step was used to eliminate sub-micron colloidal particles (NLC) interferences, the effect of spike level no longer had a significant effect on E-value (E_{resin}) with the exception of the soils that had very low carbonate contents (CW-W3; 1.00 % CO_3 and CW-G; 0.16 % CO_3).

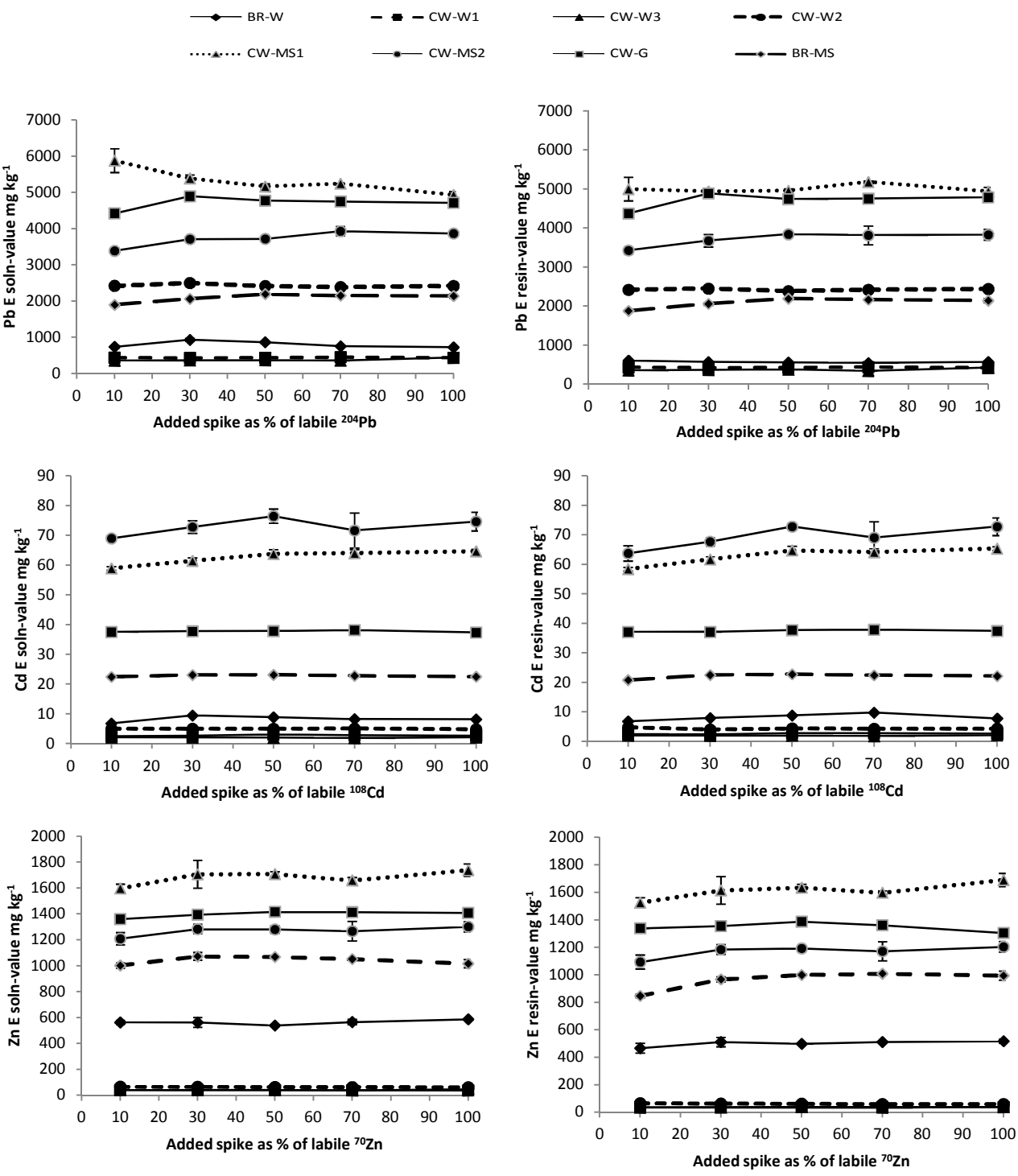

Fig. 3.5 Lead, cadmium and zinc E-values (mg kg^{-1}) assayed from isotope abundance in the solution (E_{soln}) or resin (E_{resin}) phase, as a function of isotope spike addition expressed as % labile ^{70}Zn, ^{108}Cd and ^{204}Pb for soils from Clough Wood and Black Rocks. The level of spike to be added was based on an initial estimation of E-value which, in turn, used sufficient spike to produce a 50% change in the natural abundance of the DTPA-extractable pool of ^{70}Zn, ^{108}Cd and ^{204}Pb content. The error bars show standard error of three replicates.

The same effect was seen in the case of Cd in soil CW-W1 where the effect of the spike level was no longer significant when E_{resin} was determined. Lombi et al (2003) found that NLC metals are more significant in soils with a high pH value. The small, but significant, effect of spike level detected by ANOVA for the Pb, Cd and Zn data may have arisen from a small systematic error associated with adding different amounts of the spike solution (0.1, 0.3, 0.5, 0.7 and 1.0 mL) to each soil suspension.

Hamon et al. (2008) found that increasing ^{206}Pb spike (added at 1.00, 2.00, 5.00, 10.0, 20.0, 50.0, and 100% of total soil Pb) caused an increase in E-value with increasing amount of spike isotope added to the soil. They showed that E-value was overestimated, even at relatively low additions of spike (^{206}Pb < 2% total soil Pb), and this was attributed to precipitation of the added spike. By contrast with the approach of Hamon et al. (2008), much lower concentrations of spike were added to the soil suspension in the current study. Hamon et al. (2008) applied ^{206}Pb (24.1 % natural abundance) to produce concentrations equivalent to 20, 40, 100, 200, 400, 1000 and 2000 mg kg^{-1}. The ^{204}Pb spike concentrations used in the current work were approximately 3.00, 9.00, 15.0, 21.0 and 30.0 mg kg^{-1} and these concentrations were equal to 10.0, 30.0, 50.0, 70.0 and 100% of 'labile' ^{204}Pb estimated by 0.05 M DTPA extraction rather than based on a proportion of the total soil metal content as used by Hamon et al (2008). Thus adding the isotopic spike does not appear to have caused either precipitation or pH perturbation of the system due to the small amount of spike used. Using isotopes with lower natural abundance (^{70}Zn = 0.62%; ^{108}Cd = 0.89% and ^{204}Pb =1.42%) allows very small amounts of isotope spike to be used which minimizes the impact of the spike on metal equilibrium as well as reducing the experimental cost. The current results are also consistent with the result of Sterckeman et al (2009). The authors found that adding varying amounts of ^{111}Cd spike (12.8 % natural abundance) over more than one order of magnitude

in two soils with low organic matter (1.70 %) and zero carbonate content had an insignificant effect on Cd E-value.

3.3.3.2 Effect of NLC metals on E-value: comparing E_{soln} and E_{resin}.

The Chelex-100 resin used to adsorb metals from filtered equilibrated spiked supernatant solutions should not adsorb metal from the NLC metals. This assumption may not be valid if (i) the colloid itself is absorbed by the resin and transferred to the eluent because of insufficient washing or (ii) the resin is such a powerful 'zero-sink' that it causes dissolution of non labile forms associated with NLC metals. The first exception was addressed simply by repeating the washing steps several times to remove any colloidal particles. Ma et al. (2006) measured E-value using a Chelex-100 purification step and Donnan dialysis, which separates the free metal ions in the solution phase from soluble and colloidal complexes (Nolan et al., 2003). Since the free metal ions measured by the Donnan dialysis must be fully isotopically exchangeable, this method was used by the authors as a reference for measuring an 'authentic' E-value. They found that the results of Cu E-value measured by both methods using ^{64}Cu were very close indicating that the resin purification step can be successfully used to minimize NLC metal interferences in determination of labile Cu in soils. Iminodiacetate-based chelating resins, such as Chelex-100 resin, appear to extract only free metal ions and kinetically labile forms in the aquatic systems (Florence et al., 1992, Ndung'u et al., 2003, Lombi et al., 2003), suggesting that the second exception is unlikely to present a source of error.

The effect of NLC metals on E-value can be determined by comparing values of E_{soln} and E_{resin}. The two estimates of labile metal were highly correlated ($r = 0.997$); the slope from the regression equation based on all metals studied ($E_{soln} = 1.0268\ E_{resin}$) indicates that the values of E_{soln} was, on average, 2.68% higher than E_{resin} (Fig. 3.6). Although this represents a very small difference it was significant in all soils and for all three metals ($P \leq 0.02$). This clearly suggests

that a small but significant amount of metal may be associated with NLC metals in the solution phase. The greatest differences between E_{soln} and E_{resin} were in soils with high humus contents and pH. This is in agreement with the observation that NLC metals become more mobile at high pH (Lombi et al., 2003) but also suggests a possible association with humic acid. Thus sub-micron forms of non-labile metal may occur in suspension either strongly bonded to humic/fulvic acids or occluded within $CaCO_3$ particles. Furthermore, the results suggest that measuring E_{soln} could lead to a small overestimation of the true E-value in soils with high organic matter and/or carbonate content. This may also explain some instances of relatively large variation in E_{soln} values for Pb in the woodland and calcareous minespoil soils when the soils were treated with different spike concentrations (Fig. 3.5). By contrast, the E_{resin} values in the same soils were stable across the different spike concentrations added to the soils.

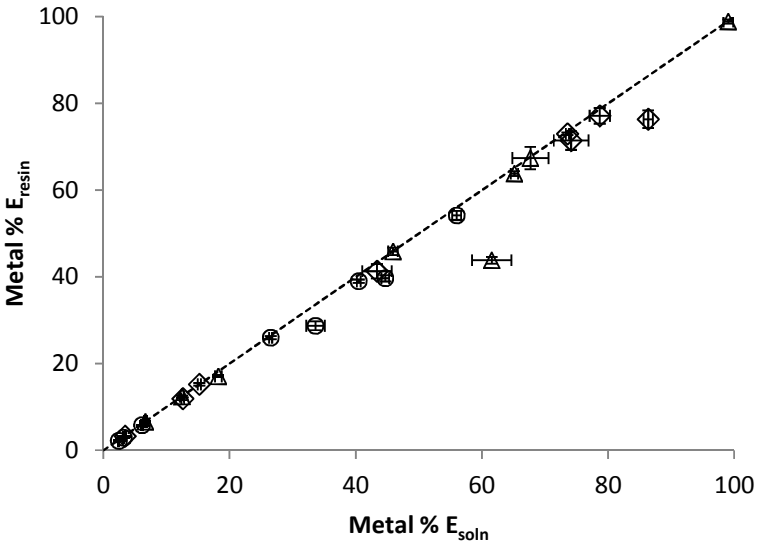

Fig. 3.6 Comparison of the isotopically exchangeable pool of Zn (○), Cd (◇) and Pb (△) measured as E_{soln} and as E_{resin} to demonstrate the extent of NLC metal interferences on metal lability. The error bars show standard errors of five replicates while dotted line represents 1 : 1 relation.

3.3.4 Labile Zn, Cd and Pb measured by single and sequential extractions compared to E-values

Although single-step extractants are frequently used to estimate the labile metal pool, results usually show large differences in the amount of Zn, Cd and Pb solubilised by each extractant. Figure 3.7 shows that the value of 'labile' Zn, Cd and Pb determined using three single extractants varied significantly for all soil samples ($p < 0.001$). All comparisons were based on the labile pool as a proportion of total metal concentration and the E-value was measured using the Chelex-100 purification step (E_{resin}).

It appears (Fig. 3.7) that E-value had no consistent correspondence to any single chemical extraction procedure. Extraction methods represent arbitrary levels of extraction power and capacity and the efficiency of individual approaches can be strongly influenced by soil characteristics (Martin et al., 1987, Hutchinson et al., 2000, Ayoub et al., 2003, Conesa et al., 2007). It was suggested by Young et al. (2005) that there is unlikely to be an exact match between E-value and any extractant. The isotopically exchangeable metal pool was considerably larger than the amount extracted by the traditional DTPA method (0.005 M) in all cases (Fig. 3.7). By contrast, 0.43 M HNO_3 and 0.05 M EDTA were reasonably similar to E-values below pH 6.36 and may be suitable for acidic (organic) woodland soils (Fig. 3.8). Groenenberg et al. (2010) suggested that extraction with EDTA or 0.43 M HNO_3 provided a convenient estimate of the labile metal pool. Metal extractions using a diluted acid (0.43 M HNO_3) have been used by several authors to estimate 'chemically reactive' metal (Gooddy et al., 1995, Temminghoff et al., 1997, Cances et al., 2003, Groenenberg et al., 2010). However, the amounts of metal extracted by HNO_3 and EDTA were significantly different ($P < 0.0001$), although there was a good correlation ($r = 0.85$) of the two values for the acidic soils (pH < 6.36). This is in agreement with Groenenberg et al (2003) , Tipping et al. (2003) and De Vries et al. (2005) who

also found good agreement between metal extracted by 0.43 M HNO_3 and EDTA in organic soils.

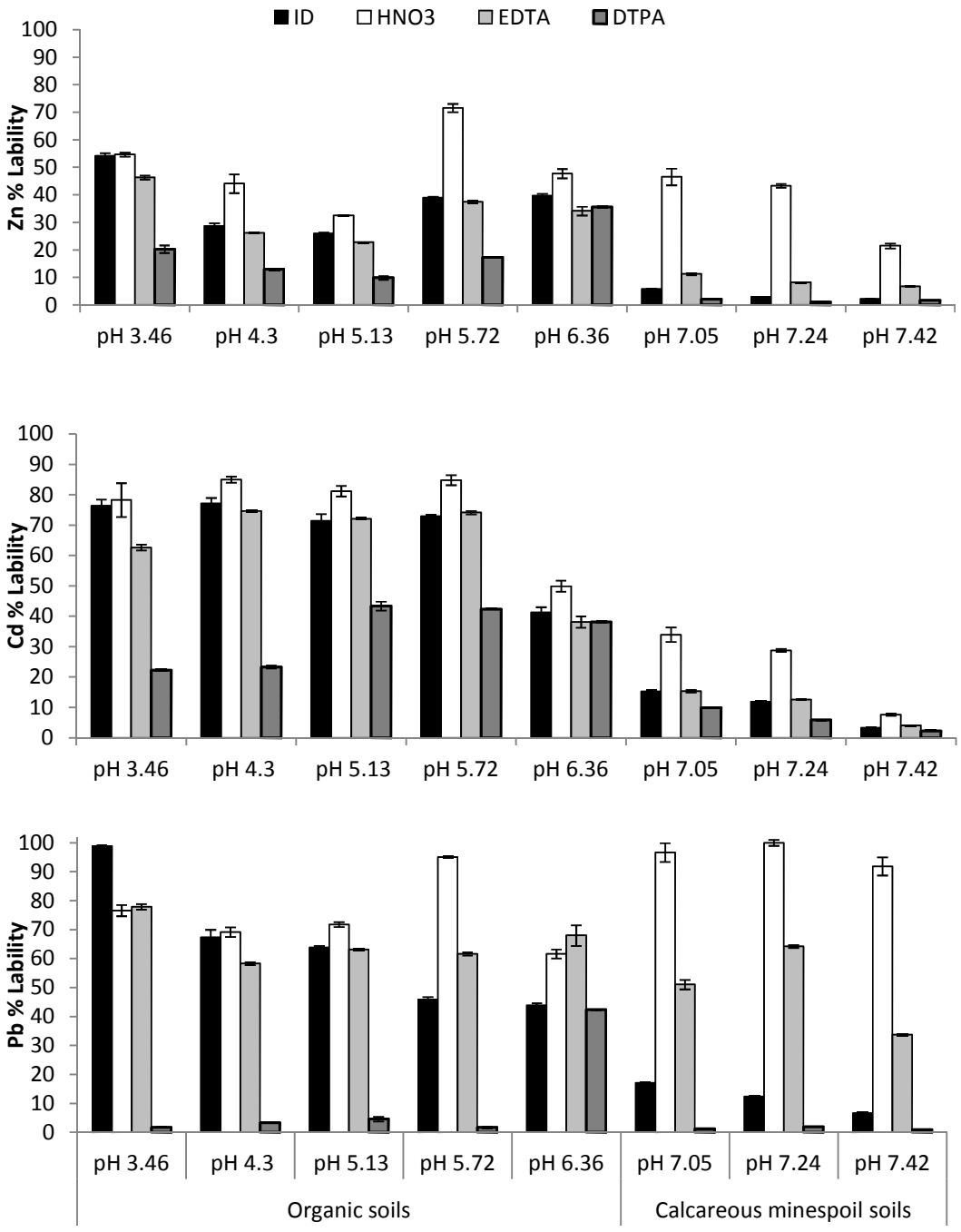

Fig. 3.7 Comparison of isotopically exchangeable pool (ID; E_{resin}) of Zn, Cd and Pb with 0.005 M DTPA, 0.05 M EDTA and 0.43 M HNO_3 under different soil pH conditions. The error bars show standard error of three replicates.

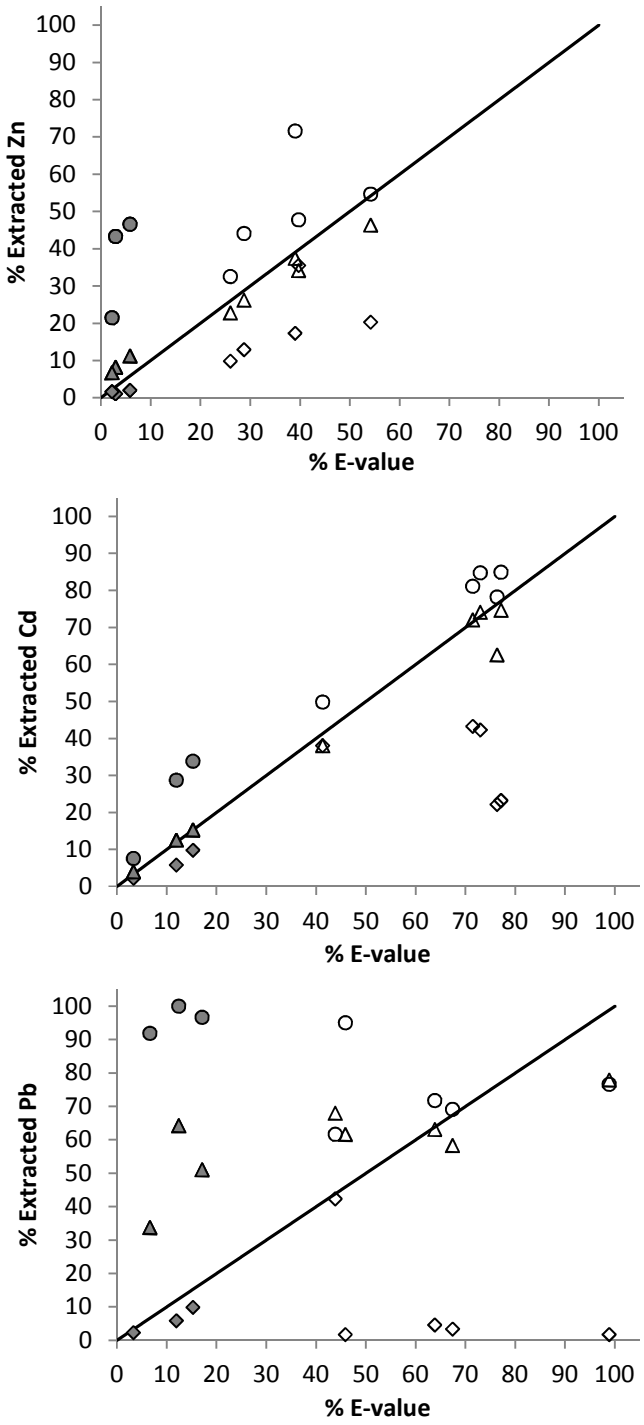

Fig. 3.8 Correlation between extracted metal by ID (%E-value) and by 0.43 M HNO_3 (○), 0.005 M DTPA (◇) and 0.05 M EDTA (△) of Zn, Cd and Pb. The open and closed symbols refer to the organic and calcareous minespoil soils, respectively where the solid line represents 1:1 relation.

3.3.4.1 Comparison with EDTA extraction

To determine the level of correspondence between the different measures of labile metal compared to E-value, the root mean square difference (RMSD, equation 3.1) across all the soil samples ($n = 8$) was used to measure the average difference between fractions (% of total) extracted by two different methods (x_i and y_i for each soil sample i), as suggested by Degryse et al. (2011):

$$\mathrm{RMSD} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - y_i)^2} \qquad (3.1)$$

The proportion (%) of Zn, Cd and Pb extracted with EDTA was significantly correlated with the % E-values (n = 8; r values: Zn = 0.99, Cd = 0.99 and Pb = 0.72; $p \leq 0.01$), and the RMSD (% total) was the lowest of all the comparisons (n = 8; RMSD values: Zn = 4.85, Cd = 5.09 and Pb = 27.2) indicating that extraction with EDTA provided the best estimate of metal E-value of all the single chemical extractants across the range of soils studied. Figure 3.8 shows the correlation between Zn, Cd and Pb extracted by single extractants and E-value in all soils. Cadmium-EDTA and Zn-EDTA were less scattered around the 1:1 line than Pb-EDTA in relation to E-values (Fig. 3.8). In the acidic soil samples from both Black Rocks and Clough Wood sites (open symbols in Fig. 3.8), the estimated value of Zn-, Cd- and Pb-EDTA corresponded well with E-values. There were no significant differences between all studied metals yielded by EDTA and E-values ($P > 0.05$) for soils with pH ≤ 6.36. However, for the most acidic soils (pH 3.46 and 4.3), lower values were obtained with EDTA extraction compared to the E-values. These results are consistent with the assumption that metal–EDTA complexes are less stable at low pH (Gabler et al., 2007), possibly due to protonation of the chelate and formation of Al-complexes.

By contrast, in the calcareous soils with pH > 7.0, EDTA extraction clearly overestimated the E-value for Zn and Pb by an average factor of 2.38 and 4.12,

respectively. Surprisingly, Cd-EDTA values were very close to the 1:1 line (closed symbols in Fig. 3.8) in the calcareous soils suggesting the absence of a Cd fraction that is EDTA-accessible but not isotopically exchangeable ($P > 0.05$). These results are in agreement with Fujii and Corey (1986) and Gabler et al. (2007) who determined Cd-E-value and Cd-EDTA in a range of soil samples and found that EDTA extraction successfully estimated the isotopically exchangeable pool of Cd. However, the EDTA extraction method is clearly inappropriate for estimating the labile metal pool of Zn and Pb in calcareous minespoil soils.

Extraction with EDTA is expected to dissolve all exchangeable and organically complexed forms of metal and a proportion of the metal bonded to iron and manganese hydroxides (Rauret, 1998) and to carbonates (Quevauviller, 1998). Therefore, the fractionation provided by the SEP may explain the deviation of Pb- and Zn-EDTA in the calcareous minespoil soils. As shown in Fig. 3.9, Pb in the calcareous minespoil soils was strongly associated with the carbonate phase (F2, c. 86 %) suggesting the possible presence of cerussite ($PbCO_3$); this is in agreement with SEP data from Li and Thornton (2001) for Derbyshire minespoil soils. Also, a correlation between Pb in the residual fraction and total phosphate-P ($r = 0.57$) may suggest the presence of Pb phosphate minerals, such as pyromorphite [$Pb_5(PO_4)_3Cl$], in these soils (Li and Thornton, 2001). The XRD results undertaken by Cotter-Howells (1991) in minespoil soils in Derbyshire, UK also showed that lead carbonate (cerussite) is one of the major Pb minerals. Therefore, in calcareous minespoil soils it is likely that dissolution of carbonates and phosphates by EDTA would release non-labile Pb occluded within these mineral phases and so the Pb extracted substantially exceeded the isotopically exchangeable Pb content (Fig. 3.7). For Zn in calcareous minespoil soils, the majority was associated with the residual fraction (F5, c. 51.8%) followed by the carbonate fraction (F2; c. 19.4) and the Fe-Mn oxide fraction (F3; c. 16.2%) of the SEP (Fig. 3.9).

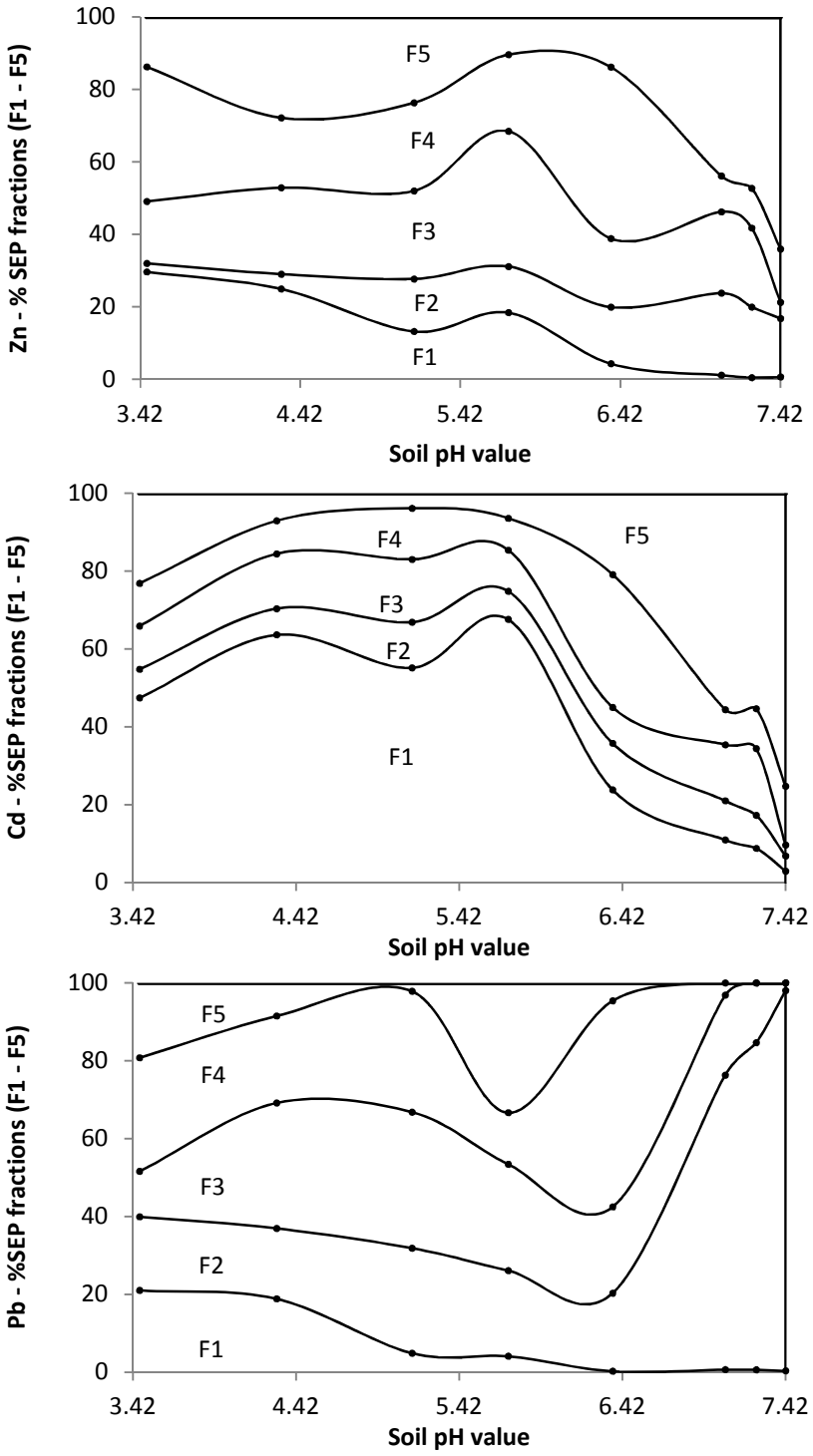

Fig. 3.9 Distribution fraction pattern of Zn, Cd and Pb in the five extracted fractions by sequential extraction procedure (SEP) of Li and Thornton (2001) under different soil pH.

Rico et al. (2009) found a significant correlation between EDTA-extractable Zn and the Zn-bound to F3 of the SEP developed by Tessier (1979).

In the current study a significant correlation was found between EDTA-extractable Zn and Zn-bound to the carbonate phase in the calcareous minespoil soils, possibly as smithsonite ($ZnCO_3$) ($r = 0.99$; $P < 0.01$). Laing (2010) reported that EDTA is assumed to dissolve carbonates along with surface-adsorbed metal. Thus, as for Pb, it is likely that EDTA would mobilize non-labile Zn by dissolution of smithsonite resulting in an overestimation of Zn E-values in the calcareous minespoil soils. Also, it is possible that EDTA partly dissolved phases such as iron and manganese oxides, thereby releasing Zn (and Pb) associated with these fractions, which would not be isotopically exchangeable. Cadmium is clearly present at much lower concentrations than Zn and Pb in the calcareous minespoil soils. This may mean that whereas weathering of the original sulphide minerals leads to precipitation of (secondary) Pb and Zn carbonates, the concentration of Cd is insufficient to produce discrete Cd-carbonates. Therefore Cd released from primary minerals may be more likely to form surface-adsorbed complexes, on both $CaCO_3$ and Fe oxides, which remain vulnerable to dissolution in EDTA and are isotopically exchangeable. This might explain the closer relationship between Cd E-value and Cd-EDTA.

3.3.4.2 Comparison with Nitric acid extraction

In general, the amount of Zn, Cd and Pb extracted by HNO_3 was higher than that of EDTA and DTPA (Fig. 3.7). Dilute HNO_3 used as an extractant is said to present a particular 'advantage' in that there is no mobilization of metals due to complex formation with the anion ligand, in contrast to extraction reagents such as EDTA, CH_3COOH or HCl (Meers et al., 2007, Tack, 2010). The choice of 0.43 M HNO_3 might arise from previous use of 0.43 M acetic acid to extract trace metals (Kennedy et al., 1997). The fraction of Zn, Cd and Pb extracted by HNO_3 was correlated quite poorly with E-values for Zn and Pb ($n = 8$; r values: Zn =

0.61, Cd = 0.98 and Pb = -0.65), and the RMSD was higher than that of EDTA for all three metals (n = 8; RMSD values: Zn = 25.2, Cd = 11.3 and Pb = 55.6). As was found for EDTA, the fraction of Zn, Cd and Pb extracted by HNO_3 corresponded reasonably well with E-values for organic soils with pH values up to 6.36 (n = 5; RMSD values: Zn = 16.8, Cd = 8.63 and Pb = 25.7) where the metal-HNO_3 value was very close to 1 : 1 line (Fig. 3.8). However, in the calcareous minespoil soils (pH > 6.36), the HNO_3 extracted fraction for all metals was again considerably larger than the E value (n = 3; RMSD values: Zn = 34.7, Cd = 14.7 and Pb = 84.5) and showed considerable scattering around the 1 : 1 line (Fig. 3.8).

Figure 3.10 shows the correlation between soil carbonate content (%) and the ratio of metal extracted by HNO_3 : E-value for Zn, Cd and Pb. The trend suggests that soils with even barely detectable levels of carbonate (c. 1%) will show a disparity between extractable and isotopically exchangeable metal contents. Thus results for HNO_3 extraction parallel those shown by EDTA extraction and confirm previous suggestions that both 0.43 M HNO_3 and EDTA are suitable for use in organic soils with low pH rather than calcareous soils (Rodrigues et al., 2010, and the references cited therein).

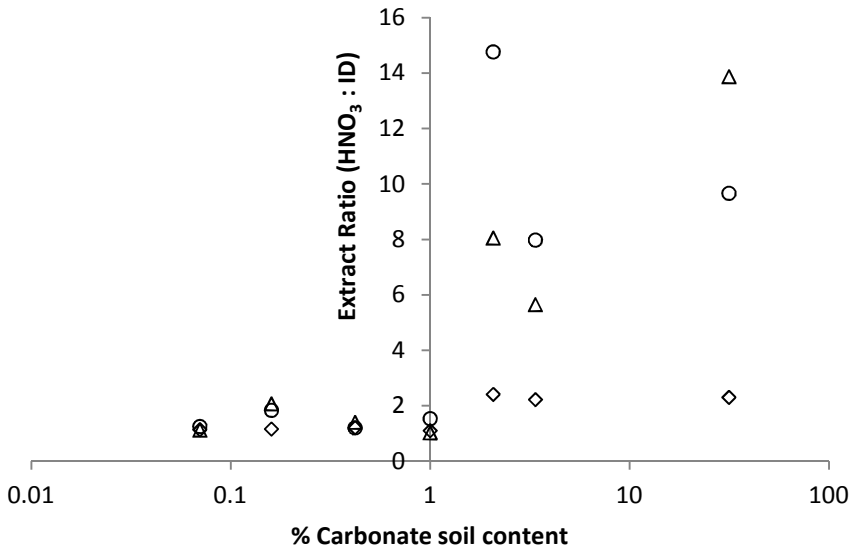

Fig. 3.10 Ratio of Zn (○), Cd (◇) and Pb (△) extracted by HNO_3 to ID (HNO_3 : ID) as a function of % soil carbonate. The X axis transferred into logarithm scale.

3.3.4.3 Comparison with DTPA extraction

Extraction with DTPA generally underestimated the E-value in all soils although there was a positive correlation and comparatively low RMSD between the two methods in the calcareous minespoil soils (n = 3; r values: Zn = 0.68, Cd = 0.96 and Pb = 0.31; RMSD values: Zn = 2.44, Cd = 4.72 and Pb = 11.5) (Fig. 3.7 and 3.8). The DTPA method has been successfully applied as an empirical prediction of plant uptake (Conesa et al., 2007, Conesa and Faz, 2011) and was originally developed for diagnosing trace metal nutrient deficiency in calcareous soils (Hooda, 2010). Therefore, it was not intended to simply dissolve all metals present as carbonates. Perhaps for this reason it gives the best estimation of the labile metal pool in the calcareous minespoil soils (BR-MS, CW-MS1 and CW-MS2). However, the DTPA method was also never intended to estimate the entire reactive fraction and so, in contrast to the EDTA and HNO_3 methods, it may generally be expected to underestimate the E-value (Fig. 3.8). Thus, its extraction capacity is particularly limited in organic soils with low pH where it

substantially underestimated the isotopically exchangeable metal pool (n = 5; RMSD values: Zn = 20.7, Cd = 38.9 and Pb = 61.3). Only in one soil (BR-W) were the E-values and DTPA-metal concentrations for Zn, Cd and Pb very close (P > 0.05). This soil was quite unusual in that it had a fairly neutral pH of 6.36 but a large LOI of 56.1%. The soil was collected from a steep slope at the Black Rocks site where it had been superficially affected by colluvial carbonate material from the adjacent calcareous minespoil soil with high carbonate content (BR-MS, 31.5% CO_3).

Overall, the best estimation of E-value by extraction with EDTA, DTPA and HNO_3, based on RMSD, followed the order EDTA > DTPA> HNO_3 for Zn and Pb and EDTA > HNO_3 > DTPA for Cd. However, it is clear that soil pH range must be considered if reactive metal concentration is to be estimated by a single extraction method.

3.3.4.4 Comparison with the SEP

The SEP protocol of Tessier et al. (1979) has been thoroughly researched and rigorously tested for metal sequential extraction (Li and Thornton, 2001, and the references cited therein). The amount of isotopically exchangeable Zn, Cd and Pb showed no consistent correspondence to any single SEP fraction (Fig. 3.11), in agreement with the results of Young et al. (2000) and Ahnstrom and Parker (2001). It was suggested by Harrison et al. (1981) that metal lability generally decreased (from high to low) in the order of the extraction sequence in SEPs. Based on RMSD values, the % E-value corresponded well with metal extracted in the combined fractions F1-F2 (the sum of the first 2 steps) for Zn and Cd in all soils (n = 8) and in the combined fractions F1-F3 (the sum of the first three steps) for Pb in organic soils (n = 5); RMSD values (r values) were: Zn = 14.9 (0.71), Cd = 8.95 (0.97) and Pb = 21.5 (0.21).

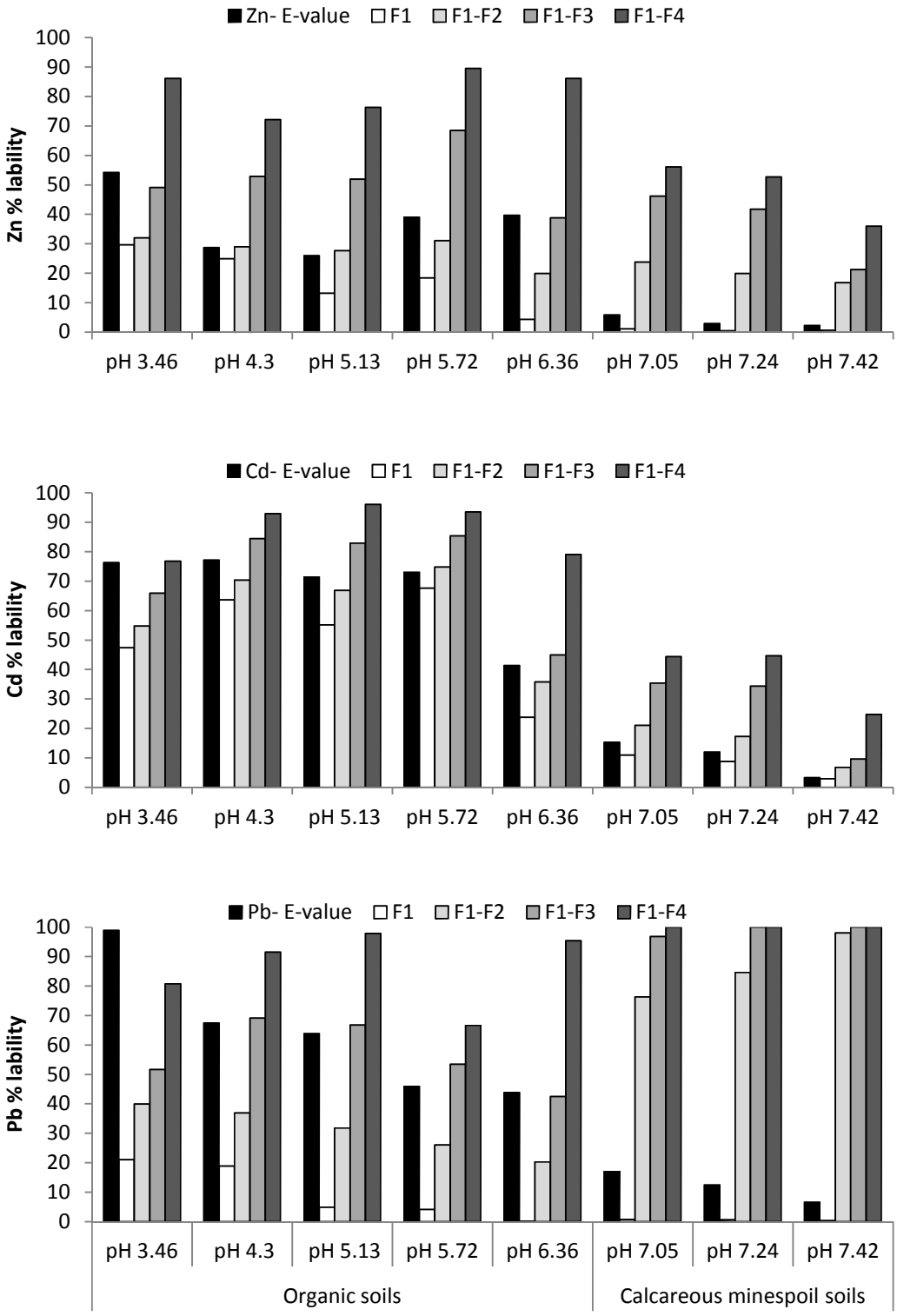

Fig. 3.11 Comparison of isotopically exchangeable pool of Zn, Cd and Pb (E-value) with different fractions of a sequential extraction procedure (SEP) of Li and Thornton (2001) under different soil pH.

In the calcareous minespoil soils there was greater correspondence with F1 alone (n = 3; r values: Zn = 0.90, Cd = 1.00 and Pb = 0.92; RMSD values: Zn = 3.20, Cd = 3.09 and Pb = 12.2). This probably arises because the addition of F2 (carbonate) includes a large amount of non-labile metal bound into solid carbonate matrices – especially in the case of Zn and Pb. By contrast, summing F1+F2 in the acidic organic soils simply adds humus-bound metal liberated by the acidic F2 extractent and thereby includes metal which would genuinely form part of the isotopically exchangeable fraction. In fact, in the organic soils with pH up to 6.36, summing F1-F3 provided the best estimation of Zn, Cd and Pb E-values (n = 5; RMSD values: Zn = 20.7, Cd = 9.61 and Pb = 21.5). The same summation of SEP fractions for the minespoil soils increased the RMSD values by a factor of 1.65, 1.85 and 4.36 for Zn, Cd and Pb, respectively. The addition of non-labile metal retained within oxides in F3 grossly overestimated E-value (Fig. 3.9 and 3.11). Huang et al. (2011) also showed that the isotopically exchangeable pool of Pb was much lower than the sum of the first three fractions of the modified sequential BCR approach. Ahnstrom and Parker (2001) found that 70-75% of Cd was 'isotopically' labile in the carbonate fraction (F2), while within the oxidizable fraction (F3), only 35-41% of the Cd was labile. It has been suggested that the sum of the first two SEP fractions (F1-F2), or occasionally including the third fraction (F1-F3), may be considered 'available to plants' or 'potentially labile' (see Ahnstrom and Parker, 2001 and references therein).

3.3.5 Variation of Zn, Cd and Pb E-values with soil characteristics

Average E-values (E_{resin}) determined for the eight tested soils (Table 3.5) ranged from 34.8 to 1600 mg Zn kg^{-1}, 1.95 to 69.2 mg Cd kg^{-1} and 372 to 5000 mg Pb kg^{-1}. The values were highly reproducible, in all cases returning an average % coefficient of variance (% CV) among replicates of 4.80 %. Expressed as a proportion (%) of total soil metal content, %E-values showed a very wide range across the study areas (CW and BR); 2.00-54.0, 3.00-77.0 and 7.00-99.0 % for

Zn, Cd and Pb, respectively and so these soils should provide a satisfactory test of the method. The total Zn, Cd and Pb concentration in the soil explained most of the variation in % E-value: r (P-values) for the three metals were Zn = -0.88 (0.004), Cd = -0.93 (0.001) and Pb = -0.87 (0.005).

Figures 3.12 and 3.13 show the variation of metal E-values as affected by soil pH and soil organic matter (represented as % LOI). The results (Fig. 3.12) indicated that the isotopically exchangeable metal pool increased with decreasing soil pH ($P \leq 0.01$) for Zn, Cd and Pb (r value: Zn = -0.81, Cd = -0.91 and Pb = -0.98) as also found by Degryse et al. (2004). Soil organic matter content also greatly affects metal lability (Ottosen et al., 2009). Positive correlations were found for % LOI and % E-value (Fig. 3.13) for Zn ($r = 0.79$; $p = 0.02$), Cd ($r = 0.46$; $P = 0.25$) and Pb ($r = 0.60$; $P = 0.12$) suggesting that metals bonded to humic and/or fulvic acids are predominantly labile. In the organic soils with $pH \leq 6.36$, the measured dissolved organic carbon (DOC) in the 0.01 M $Ca(NO_3)_2$ (data not shown) also showed a positive significant correlation with %E-value for Zn ($r = 0.96$; $p = 0.0001$), Cd ($r = 0.69$; $P = 0.05$) and Pb ($r = 0.83$; $P = 0.01$).

Table 3.5 Isotopically exchangeable metal content (E-value) and proportional lability (% total metal) for Pb, Cd and Zn assayed from isotope abundance in the solution (E_{soln}) or resin (E_{resin}) phases in soil suspensions from Clough Wood and Black Rocks. Values are the average of five replicates ± standard error of the mean

	Solution Phase		**Resin Phase**	
	E_{soln} (mg kg^{-1})	% Total	E_{resin} (mg kg^{-1})	% Total
Lead				
BR-W	799 ±40.4	61.5	569 ± 10.4	43.9
CW-W1	434 ± 3.96	65.1	426 ± 3.65	63.8
CW-W3	374 ± 15.9	67.7	372 ± 14.3	67.4
CW-W2	2430 ± 18.9	99.0	2420 ± 11.4	98.9
CW-MS1	5320 ± 157	18.2	5000 ± 46.0	17.1
CW-MS2	3720 ± 93.9	12.4	3720 ± 78.5	12.4
CW-G	4700 ± 78.1	45.9	4710 ± 87.9	45.9
BR-MS	2070 ± 52.2	6.60	2090 ± 56.7	6.62
Cadmium				
BR-W	8.28 ± 0.45	43.3	8.23 ± 0.50	43.0
CW-W1	2.71 ± 0.10	74.1	2.63 ±0.08	71.5
CW-W3	2.00 ± 0.04	78.6	1.96 ± 0.05	77.1
CW-W2	4.92 ± 0.04	86.3	4.35 ± 0.12	76.3
CW-MS1	62.6 ± 1.06	15.2	62.9 ± 1.27	15.3
CW-MS2	72.9 ± 1.28	12.6	69.2 ± 1.70	11.9
CW-G	37.7 ± 0.14	73.5	37.5 ± 0.16	73.0
BR-MS	22.8 ± 0.14	3.40	22.1 ± 0.34	3.30
Zinc				
BR-W	562 ± 7.66	44.7	499 ± 9.14	39.7
CW-W1	37.8 ± 0.34	26.5	37.1 ± 0.52	26.0
CW-W3	40.7 ± 1.77	33.6	34.8 ± 1.26	28.7
CW-W2	62.7 ± 0.80	56.0	60.7 ± 1.17	54.2
CW-MS1	1680 ± 24.7	6.09	1610 ± 26.8	5.84
CW-MS2	1270 ± 15.8	3.18	1170 ± 19.7	2.93
CW-G	1400 ± 10.4	40.4	1350 ± 13.6	39.0
BR-MS	1040± 14.2	2.41	962 ± 29.9	2.22

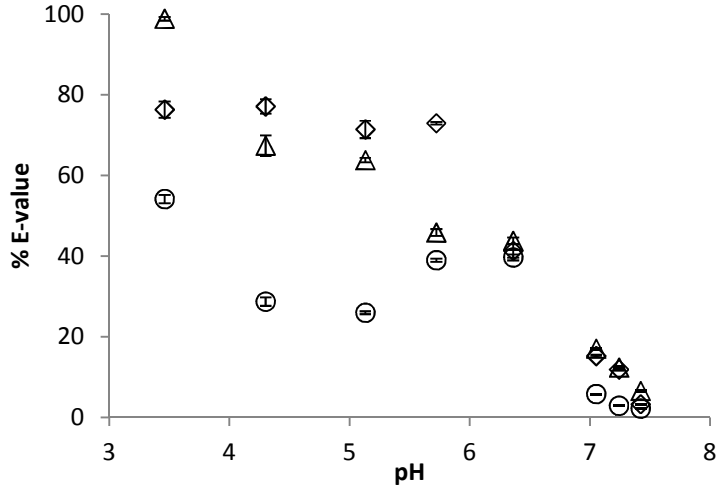

Fig. 3.12 Lability (%Eresin) of Zn (○), Cd (◇) and Pb (△) as a function of soil pH. The y error bars show the standard error of five replicates.

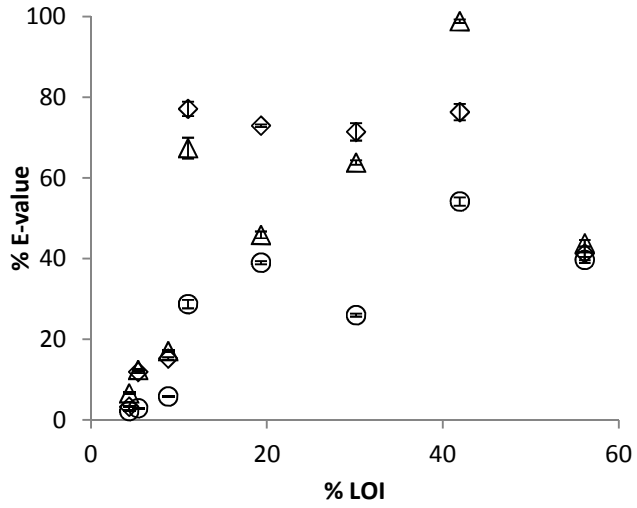

Fig. 3.13 Lability (%E_{resin}) of Zn (○), Cd (◇) and Pb (△) as a function of soil %LOI. The y error bars show the standard error of five replicates.

The soils based directly on calcareous minespoil, with high metal contents (CW-MS1, CW-MS2 and BR-MS; Tables 3.2 and 3.5) showed the lowest lability of Zn, Cd and Pb with average %E-values of 3.66, 10.0 and 12.0%, respectively. This probably reflects a combination of 'source characteristics' and soil properties. 'Minespoil' soils may retain primary and secondary mineral sources (sulphides and carbonates, respectively) and their large metal contents and calcareous nature will facilitate the formation of secondary minerals such as mixed carbonates (e.g. $Ca_{(1-n)}Cd_nCO_3$). In addition, all these soils had higher pH values (> pH 7) and lower organic matter contents than that of the woodland and grassland soils (Table 1.3). It is likely therefore that adsorbed metal ions will be bound more strongly, probably to carbonates and Fe & Mn oxides, rather than humus as shown from SEP results (Fig. 3.9), which may progressively decrease their apparent 'lability' by occlusion; soil physiochemical properties have been shown to affect metal lability (Nakhone and Young, 1993, Young et al., 2000, Degryse et al., 2004, Vega et al., 2004, Oliver et al., 2006, Sterckeman et al., 2009, Degryse et al., 2011). The same degree of lability (%E-values) has been observed in the same sites used here (Black Rocks and Clough Wood) for 'radio-labile' Cd by Nakhone and Young (1993). Different ranges of metal lability were reported by Young et al. (2000) in soils historically contaminated by sewage sludge and minespoil suggesting that the origin of the contaminant influences the degree of metal lability. The acidic organic soil samples (Table 3.5) were typically from areas around the minespoil heaps, which might have been subject to marginal metal deposition as dust or smelter output, so that metals would be more labile than in the minespoil soils following an extended period of weathering (Egli et al., 2010). Thus the average lability of Zn, Cd and Pb in both woodland and grassland soils (37.5, 67.8 and 64.0%, respectively) was higher than in the calcareous minespoil soils (3.67, 10.2 and 12.0% respectively). Surprisingly, the %E-value of Zn was almost half that of Cd in all soils (Table

3.5) and this is unlikely to be due to secondary formation of Zn carbonates in the acidic soils.

Although there is an obvious influence of each soil characteristic (eg pH, %LOI, metal origin material) on the metal lability, it is indeed difficult to separate or de-couple all these factors to provide a distinctive explanation of the variation metal E-values.

4. CHAPTER FOUR: ZINC, CADMIUM AND LEAD SOLUBILITY AND LABILITY IN SOILS CONTAMINATED BY HISTORICAL MINING

4.1 OVERVIEW

Elevated metal concentrations in soils resulting from past mining activities pose significant risks to human and ecosystem health (Gleyzes et al., 2002, Tongtavee et al., 2005, Igwe and Abia, 2007, Katanda et al., 2007, Lofts et al., 2007). Contamination of metals in soil occurs in a wide range of chemical forms and fractions in the solid phase and complex species in the soil solution. Generally, these can be categorized into four major compartments (Fig. 4.1) which can be described as '(1) inert', '(2) non-labile', '(3) labile' and '(4) solution' forms. In the solid phase there are adsorbed metal ions held by surface ligand groups on minerals and soil organic matter (compartment 3) , occluded within secondary minerals and Mn and Fe oxides (compartment 2) and strongly associated with the lattice of primary minerals as an 'inert' pool (compartment 1). In the solution phase metals exist as free ions, organic, inorganic and colloidal nano-particulate complexes (compartment 4) (see Fig 4.1).

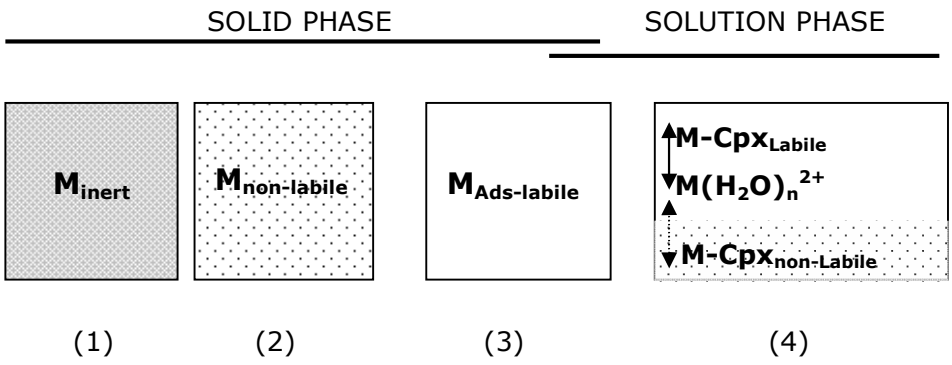

Fig. 4.1 Schematic description of metal forms in the soil-solution system nominally divided into four compartments.

Equilibrium between (3) and (4) is often regarded as essentially 'instantaneous' and represents the 'labile' metal pool (Young et al., 2005) but there may also be 'non-labile nano-particulate' metal forms suspended in the solution phase (4). Slowly reversible reactions between compartments 2 and 3 may convert the labile adsorbed metal ($M_{Ads\text{-}labile}$) to non-labile metal ($M_{non\text{-}lab}$) (fixed), and vice versa, perhaps influenced by changes in soil properties (eg pH and Eh), as a function of time. Thus, compartments 2 and 3 in the solid phase represent a reservoir of metal which can resupply trace metals to the solution phase in response to depletion by plant uptake or leaching. The first compartment (1) represents the reservoir of the 'inert' metal (M_{inert}) and has limited significance with respect to mobility and bioavailability. Both solubility and lability are likely to be affected by the original form of metal added to soil and by soil properties such as pH, organic matter content, total metal concentrations, Fe and Mn oxides, and redox potential.

In the work described in this Chapter, 246 soil samples were collected from an old minespoil area and adjacent managed grasslands, moorlands and woodlands close to Rookhope in the Weardale catchment, North Pennines. Weardale has been the site of extensive Pb, Zn and F mining since the 16th century. As a result of contamination from mining and ore processing, there are serious concerns regarding possible Zn, Cd and Pb toxicity to wildlife, domestic animals and possibly human populations. The objective of the current study is to quantify the chemically reactive pool in soils using the multi-element stable isotope dilution approach (ID), developed in Chapter 3. It was hoped that an integrated study of the three ore-body metals (Cd, Zn and Pb) in diverse ecosystems from the whole catchment would increase our understanding of the soil factors and land uses that influence metal solubility and lability. In addition, the application of the geochemical models WHAM(VI) to predict solubility in 'whole soils' was tested

using concentrations of either total or isotopically labile metal as input variables. The three aims of this chapter are listed below.

(i) Quantify Zn, Cd and Pb lability, using the multi-element stable isotope dilution approach, in a diverse range of soil ecosystems from within the catchment of an old Pb/Zn mining area and assess the controlling influences of soil properties.

(ii) From (i), develop predictive algorithms for metal lability from simple soil properties (such as pH, organic matter, and total metal content).

(iii) Assess the capability of WHAM(VI), parameterized by total or isotopically exchangeable metal content, to predict metal solubility in a wide range of contaminated soils.

4.2 MATERIALS AND METHODS

Soil chemical analyses undertaken in this chapter are described in detail in Chapter 2. The following information describes the study area, sampling procedures and some additional methods not previously described.

4.2.1 Sampling site description

Rookhope catchment lies within grouse moorlands in the N.E of England (54^{o} 45' 10" N; 2^{o} 06' 53" W) at an elevation of approximately 600 to 540 meters (Banks and Palumbo-Roe, 2010) (Fig. 4.2 and Plates 4.1 and 4.2). The area features a relatively treeless landscape primarily given over to hill farming. However, near to Allenheads Village (54^{o} 48' 13" N; 2^{o} 13' 12" W), the upper moorlands are occupied by woodlands (Fig. 4.2 and 4.3). The lower downstream vegetation (managed and unmanaged grasslands) provides rough grazing for cattle and sheep. The study area is characterized by abandoned quarries and mine workings (Plate 4.1) due to extensive Pb, Zn and F mining since 16th century.

Although evidence exists for Roman mining, the upper Wear valley has certainly been a centre of mining activities for the last 400 years (1665–1985) (Shepherd et al., 2009). Sixteen examples of old mines and three locations of mine waste have been identified within the catchment (Fig. 4.2 and see Plate 4.2 for example of mine wastes managed for cultivation). More recently, and due to the demands of steel making, fluorspar has been the principal mineral to be exploited in the centre of the catchment. Fluorspar mining continued until 1999, when the Groverake Mine (Plate 4.1) closed (Banks and Palumbo-Roe, 2010). The Rookhope catchment consists of an area of 37 km^2. Average annual precipitation reaches ~1000 mm calculated for Eastgate Village (Banks and Palumbo-Roe, 2010). The study area features two zones, based on elevation, which reflect the underlying geology (Fig. 4.4):

(i) the upland moorlands underlain by Carboniferous Limestone Series (shales and sandstones) with interbedded limestone at the lower end of the first zone;

(ii) downstream, the lower catchment is underlain by the Coal Measures constituting a thick sequence of shales, sandstones and coal veins that lie stratigraphically above the Carboniferous lithologies.

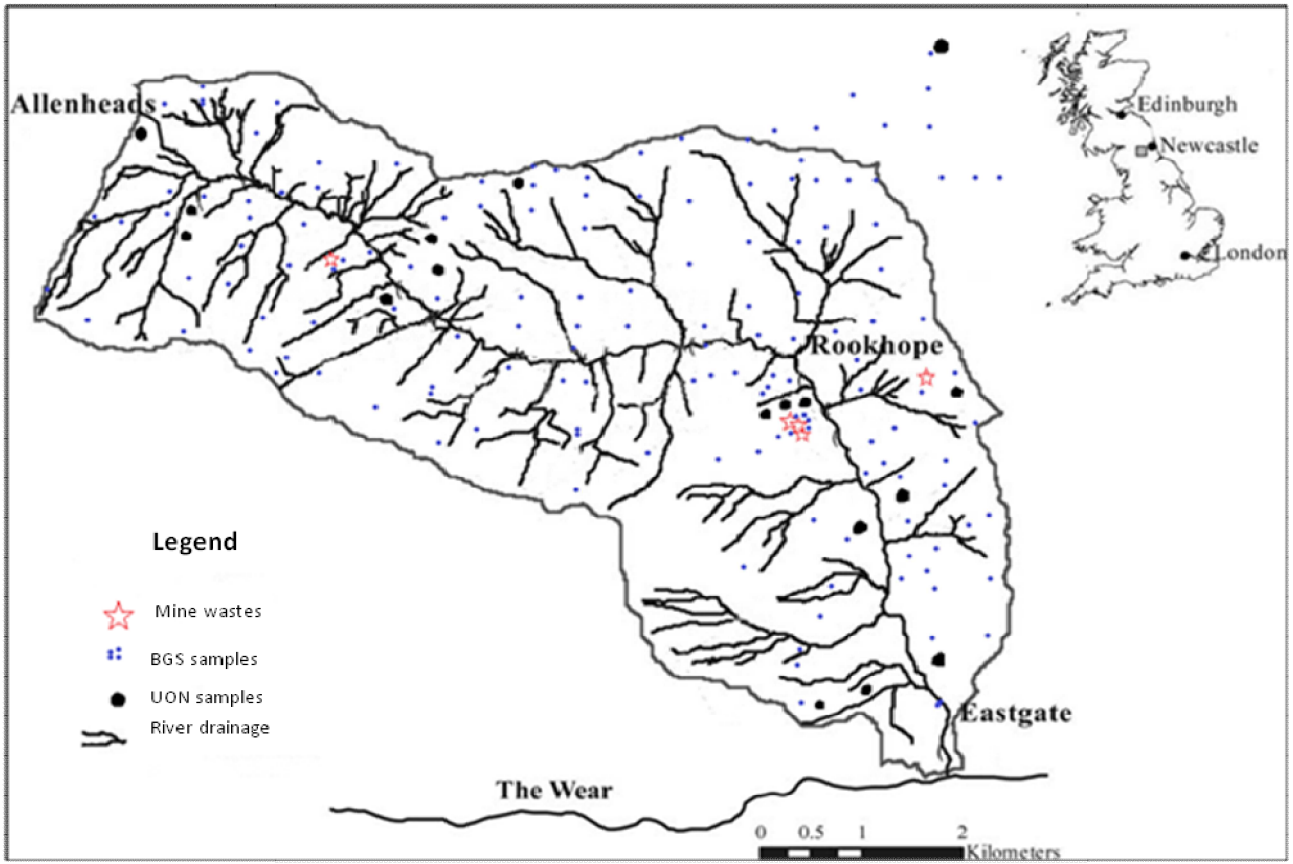

Fig. 4.2 Rookhope in Weardale catchment, North Pennine, showing the sampling points used by BGS and UoN (see text for details), (adapted from Banks and Palumbo-Roe, 2010).

Plate 4.1 Upper view of Rookhope catchment in Weardale, North Pennine. The Groverake mine is located in the centre of the picture, between the villages of Rookhope and Allenheads, and is connected underground with the nearby Frazer's Hush mine. The Groverake mine is near the convergence of three major lead-bearing veins.

Plate 4.2 The left picture shows managed and cultivated mine wastes close to Rookhope village (NY 93916\ 42432). The mine wastes were mechanically spread to cover a steep slope leading to the River Wear. The right picture shows an area of peat soils (moorlands) at the northern upper side of the catchment between Rookhope and Allenheads villages (NY 90156\ 44783).

Fig. 4.3 A satellite view of Rookhope catchment. *Source*: ''Weardale, North Pennine'' (54° 45' 22.01'' N and 2° 13' 10.38'' W). Google Earth, updated on 09/12/2009; accessed on 19/05/2011.

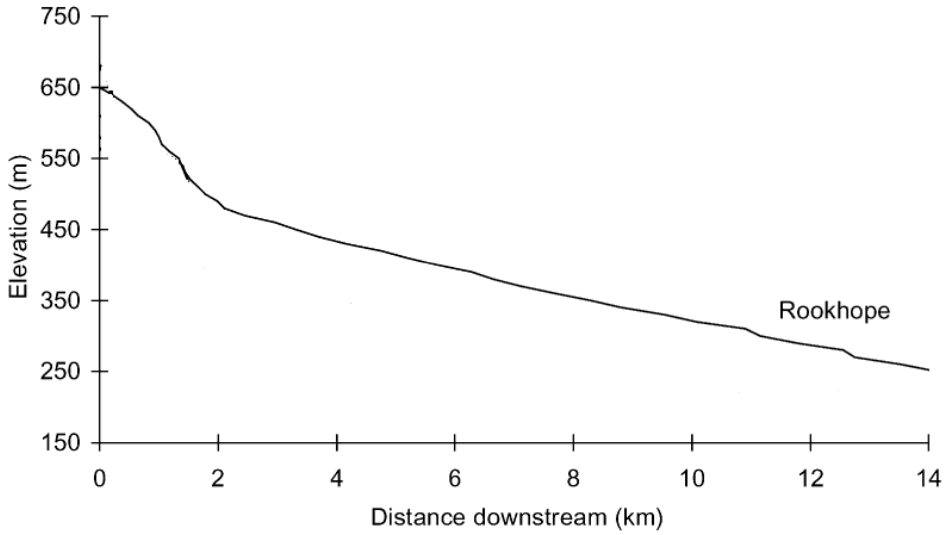

Fig. 4.4. Long profile of Rookhope in Weardale catchment. Adapted from Warburton et al. (2002).

4.2.2 Sampling procedures and analysis

The British geological Survey (BGS), Keyworth, Nottingham, UK undertook soil sampling covering the whole Rookhope catchment (Fig. 4.2) for a geochemical

survey project. BGS collected a total of 165 soil samples from the study area. Each sampling point (0-15 cm) (blue points in Fig. 4.2) was taken from five auger borings at the four corners and centre of a square with a side length of 10 meters using a standard hand auger and combined to form a bulk sample weighing around 0.5 kg. All soil samples were air-dried and disaggregated and sieved to < 2 mm. The soil samples were digested according to the BGS standard methods and analyzed for a broad range of metal concentrations using ICP-AES. The soil pH was measured in 0.01 M $CaCl_2$ suspension with a solid to solution ratio of 1:2.5 according to the standard methods of BGS (Taylor et al., 2005). Results for total metal concentrations, pH and % LOI were provided by BGS. In addition, a sub-sample of 10 g from the collected soil samples (n = 161; 4 samples were missing) were recovered from BGS in order to measure the isotopically exchangeable pools of Zn, Cd and Pb as described in section 2.2.2. Furthermore, because the total Cd concentrations measured by BGS using ICP-AES were often below the detection limit, the soil acid digest solutions were also recovered from BGS to (re-)measure total metal concentrations by ICP-MS at the University of Nottingham (UoN), as described in section 2.1.2. There were 33 soil acid digest solutions missing so it was also decided to re-digest the relevant soil samples and determine the total metal concentration according to the methods described in section 2.1.2.

To extend the study, and focus on a range of particular soil ecosystems, the sampling location was revisited again for a second sampling programme. An initial desk study was used to identify likely potential point source contributions, utilising the dataset and reconnaissance visits of BGS. The sampling protocol employed followed that used by BGS but, crucially, *without* mixing the five subsamples from the 10 m x 10 m square sampling support. The objective was to assess the local variability and also to avoid mixing of dissimilar soil materials when measuring solubility and labile metal content. A total of 85 soil samples were collected from 17 sampling points (black points refer to UoN samples in Fig.

4.2). The samples were air dried and sieved to pass through a 2 mm sieve. The soil chemical analysis was undertaken as described in section 2.1.2. However, soil pH was measured as described by the method used by BGS (soil: 0.01 M $CaCl_2$ ratio of 1:2.5).

4.2.3 Measuring Zn, Cd and Pb E-values

Duplicate suspensions of 1.0 gram of soil (< 2 mm) suspended in 30 ml 0.01 M $Ca(NO_3)_2$ were used to measure the isotopically exchangeable pool of Zn, Cd and Pb as described in section 2.2.2. A range of representative soil samples (42) were initially used to estimate E-values in order to determine the spike concentration and amount of isotope required to cover all samples. A 0.40 mL aliquot of the working spike solution was used for the whole dataset (BGS and UoN samples) with concentrations ranging from 14.7-0.879 mg $^{70}Zn\ L^{-1}$, 0.219-0.0226 mg $^{108}Cd\ L^{-1}$ and 117-9.97 mg $^{204}Pb\ L^{-1}$. According to the initial E-value results, the soil samples were divided into six groups depending on soil properties (pH and %LOI) and the samples in each group were spiked with 0.4 mL from a multi element spike solution prepared freshly from the isotope stock solutions (see section 2.1.1). The spike solution added to all the soil suspensions was equivalent to approximately 10 to 100 % change in the natural abundance of the labile pool of ^{70}Zn, ^{108}Cd and ^{204}Pb content based on the initial estimated E-values and the soil total metal content. The 'non-labile' nano-particulate metal concentration that might exist in the solution phase was measured using the Chelex-100 purification step as described in section 2.2.2.

4.3 GEOCHEMICAL MODELLING OF METAL SOLUBILITY BY WHAM(VI)

Model input and output file was described in section 2.4.

4.4 RESULTS AND DISSCUSION

4.4.1 General soil characteristics

4.4.1.1 Soil pH, LOI and trace metal content:

The descriptive statistics for elemental concentrations, pH and % LOI in the Rookhope dataset are summarized in Table 4.1; results show that the soil samples cover a wide range of characteristics. Soil pH_{CaCl2} ranged from extremely acidic to calcareous conditions, between 2.58 and 7.58. Table 4.2 shows the percentage of the total number of soils (n = 246) falling in different soil pH ranges; approximately 50 % of the soil samples had pH ≤ 4.0. Many highly acidic soils were associated with moorlands (NY87752/ 44509) with pH values less than 3.0. However, soil pH may also have been affected by the presence of pyritic minerals in the case of the most acidic soils (pH = 2.58 – 2.99) (Tack, 2010). The alkaline soils with pH ≥ 7.00, representing 6.50 % of all samples, (Table 4.2) were associated with the managed and cultivated mine wastes (NY93947/ 42454) which contained carbonate minerals. Table 4.3 shows the correlation matrix for different soil characteristics. The Pearson correlation coefficients demonstrated a significant correlation between soil pH and all measured metals and soil organic matter (represented as % LOI) with P-value < 0.01. The strongest positive correlations (r) between soil pH and total metal concentrations were found in the following order: Ca > Cu > Pb > Zn > Fe > Cd (Table 4.2). This might be explained by the presence of these metals as carbonates minerals ($CaCO_3$, $PbCO_3$, $ZnCO_3$, $CuCO_3$ and $CdCO_3$) or at least the co-existence of ore minerals with limestone.

Table 4.1 Descriptive statistical relationships for soil metal concentrations (mg kg^{-1}), soil pH and % LOI (n = 246). Data are arranged in descending order of mean value.

Soil variable	***Mean***	***Median***	***Std Deviation***	***Minimum***	***Maximum***
Fe	24400	20900	23400	1040	173000
Ca	13100	1650	35400	67.9	198000
K	7990	6800	6780	350	37000
Pb	1250	353	2500	19.9	16000
Mn	1020	220	3280	2.33	45800
Ti	455	458	313	4.45	1260
Zn	255	48.9	625	9.99	4630
Ba	130	125	76.6	17.5	615
V	36.1	34.6	20.7	2.45	93.3
Cr	33.1	30.8	21.6	1.55	100
Cu	20.4	11.3	34.5	1.79	256
Ni	13.4	10.4	11.4	0.68	85.8
Co	6.85	4.05	12.7	0.35	164
U	1.58	1.64	0.858	0.108	7.07
Cd	0.97	0.346	2.18	0.056	18.6
Mo	0.73	0.66	0.42	0.099	3.62
pH	4.24	3.89	1.22	2.58	7.58
% LOI	27.0	13.8	27.5	3.40	96.1

Table 4.2 The frequency of occurrence (%) of soil samples (n = 246) in different ranges of soil pH.

pH range	Percentage occurrence (%)
pH < 3.00	9.35
pH 3.00 - 3.99	44.7
pH 4.00 – 4.99	21.5
pH 5.00 – 5.99	14.6
pH 6.00 – 6.99	3.25
pH ≥ 7.00	6.50

Soil organic matter (as % LOI) ranged from 3.40 to 96.4 % where, in general, the least amount of organic matter was associated with relatively high soil pH and vice versa. Overall, there was a significant negative correlation of organic matter content with most of the trace metal concentrations (Table 4.3). This negative correlation could reflect the distinction between uncontaminated moorland soils and contaminated minespoil soils with lower humus contents.

Soil metal concentrations ranged from 9.99-4630 mg Zn kg^{-1}, 0.056-18.6 mg Cd kg^{-1}, and 19.9-16,000 mg Pb kg^{-1}, reflecting the degree of historical exposure to smelter fallout and minespoil. The soil samples represent a large variation in metal concentration values as shown by the range (minimum and maximum values) and also the standard deviation across all measured metals (Table 4.1).

Table 4.3 Correlation matrix for the measured metals, pH and % LOI of the studied soil samples of Rookhope catchment (n = 246).

	Ca	*V*	*Cr*	*Mn*	*Fe*	*Co*	*Ni*	*Cu*	*Zn*	*Cd*	*Pb*	*pH*
V	-0.158											
	0.015											
Cr	-0.111	**0.975**										
	0.087	*0.000*										
Mn	**0.276**	-0.002	0.007									
	0.000	*0.970*	*0.920*									
Fe	**0.523**	**0.304**	**0.333**	**0.593**								
	0.000	*0.000*	*0.000*	*0.000*								
Co	0.105	**0.181**	**0.187**	**0.895**	**0.510**							
	0.107	*0.005*	*0.004*	*0.000*	*0.000*							
Ni	**0.192**	**0.520**	**0.538**	**0.519**	**0.524**	**0.611**						
	0.003	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*						
Cu	**0.895**	-0.038	0.010	**0.230**	**0.468**	0.124	**0.253**					
	0.000	*0.561*	*0.875*	*0.000*	*0.000*	*0.056*	*0.000*					
Zn	**0.846**	-0.120	-0.077	**0.539**	**0.655**	**0.327**	**0.331**	**0.732**				
	0.000	*0.063*	*0.233*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*				
Cd	**0.656**	-0.141	-0.107	**0.727**	**0.599**	**0.524**	**0.392**	**0.559**	**0.928**			
	0.000	*0.030*	*0.100*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*			
Pb	**0.944**	**-0.206**	**-0.155**	**0.282**	**0.496**	0.108	**0.183**	**0.852**	**0.826**	**0.659**		
	0.000	*0.001*	*0.017*	*0.000*	*0.000*	*0.095*	*0.005*	*0.000*	*0.000*	*0.000*		
pH	**0.679**	**0.182**	**0.168**	**0.311**	**0.526**	**0.250**	**0.405**	**0.649**	**0.614**	**0.482**	**0.623**	
	0.000	*0.005*	*0.009*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	
% LOI	**-0.200**	**-0.535**	**-0.540**	-0.081	**-0.327**	-0.140	**-0.281**	**-0.152**	**-0.178**	-0.055	-0.110	**-0.416**
	0.002	*0.000*	*0.000*	*0.205*	*0.000*	*0.031*	*0.000*	*0.018*	*0.006*	*0.387*	*0.090*	*0.000*

*Italic and bold figures represent P and significant values, respectively.

Figure 4.6 shows only minor differences between the measurements of pre-digested solutions (open symbols) undertaken at UoN and BGS which were within the error expected as a result of using different standard solutions and instruments. There was also good agreement with the samples that were re-digested and analyzed by UoN (closed symbols, Fig. 4.6), confirming the overall reproducibility of the digestion protocol and analysis undertaken in both labs. Only the UoN results for Cd concentrations were poorly matched with the BGS

results and so all Cd analysis presented in this Chapter were from UoN measurements.

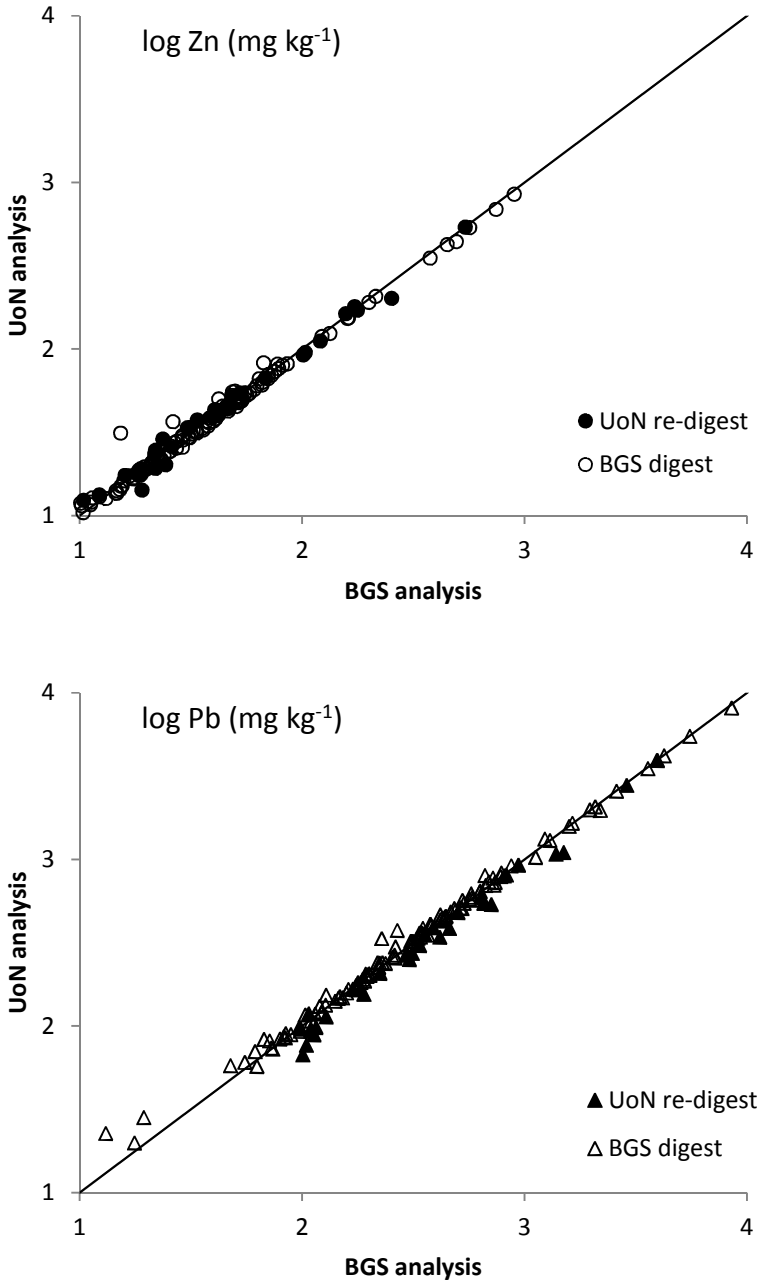

Fig. 4.5 Comparison measured metal concentrations (mg kg^{-1}) using ICP-MS at University of Nottingham (UoN) against ICP-AES at the British Geological Survey (BGS) for Zn (○) and Pb (△) (n = 161). The open symbols refer to the soil acid digest solutions produced by BGS and re-analyzed by UoN (n = 128) whereas closed symbols refer to the samples that were re-digested and re-analyzed by UoN (n = 33) as described in the text (see section 4.2.2). The solid lines represents the 1 : 1 relation.

4.4.1.2 Correlation and cluster analysis:

Table 4.3 shows the correlation between trace metals in all studied samples (n = 246). These correlations are supported by cluster analysis presented as a dendrogram (Fig. 4.7). The cluster analysis suggests two main groups. The first one includes two sub-clusters: 'Ca, Pb, Cu' and 'Fe, Zn, Cd'. The second one also includes two sub-clusters: 'V, Cr' and 'Mn, Co, Ni'. The highly significant correlation of Cr, Fe and V (see also Table 4.3) might be explained by mutual isomorphic substitution of Cr^{3+} and V^{3+} for Fe^{III} in Fe oxides; ferric iron has a similar radius (0.067 nm) and geochemical properties to Cr^{3+} and V^{3+} (Evans and Barabash, 2010, Ma and Hooda, 2010). A similar explanation can be offered for Co and Mn where adsorption of Co^{2+} can be followed by oxidation to Co^{3+} and isomorphic substitution for Mn in Mn oxides (McLaren et al., 1986, Ma and Hooda, 2010). A highly significant correlation was also found between Cd and Zn ($r = 0.93$, $P < 0.001$), presumably due to isomorphous substitution of Cd for Zn in the sulphide ore mineral sphalerite (Nakhone and Young, 1993). The strong correlation/grouping of Ca and Pb suggests the Pb ore (galena) is associated with limestone within the catchment.

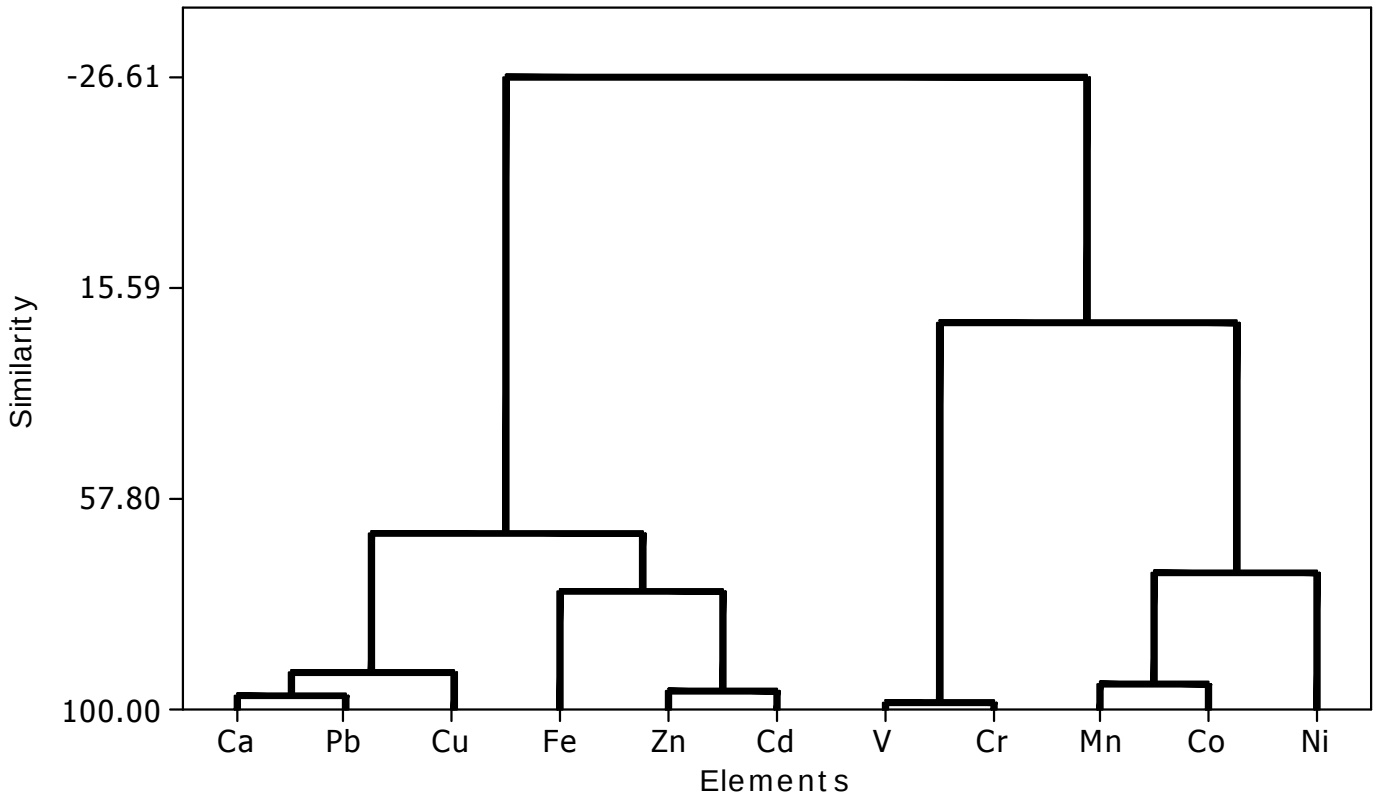

Fig. 4.6 Dendrogram obtained by cluster analysis of trace metal content in soils collected from Rookhope, Weardale, North Pennine (n = 246).

4.4.1.3 Variation in metal concentrations within each sampling location:

Analysis of the five individual soil samples collected from each sampling location within the Rookhope catchment, as described in section 4.2.2, demonstrated the variability of trace metal concentrations within the material normally aggregated for analysis. The results showed significant variation ($p < 0.001$) in the measured metal concentrations among the five sub-samples in all sampling locations. To quantify the localised variation (within the five sub-samples), the relative variability was compared based on the coefficient of variance (%CV) of the mean value (Fig. 4.7).

Coefficients of variance calculated at each location ranged from less than 10% to greater than 100%. Although the soil conditions might play some role in retaining metals to different degrees, it is likely that the highly variable deposition of mine waste must inevitably be the main factor that creates variability ***within*** the 'sampling support' used by BGS in their geochemical surveys. Fig. 4.7 shows that less variability occurred in the soils GL_7 (NY 94970/ 38968), GL_10 (NY 93829/ 39087) and GL_6 (NY 94664/ 41377). These fields were managed for cattle grazing and therefore it is likely that they were not used for disposal of minespoil and that they have been uniformly managed and so tend to be more homogeneous than surrounding areas of natural vegetation located near mining areas.

For simple geochemical mapping purposes, the strategy of aggregating five subsamples from a 10 m x 10 m square sampling support is necessary to avoid bias from the highly localised variation which is typical in land subject to minespoil dumping. However, it is equally clear that studies of solubility, metal lability or fractionation using sequential extraction procedures must inevitably be compromised by this approach to soil sampling. Aggregating soil samples from catchments subject to such localised variation in soil type and contaminant load

must lead to mixing of highly dissimilar materials which will produce unrepresentative soil metal reactions.

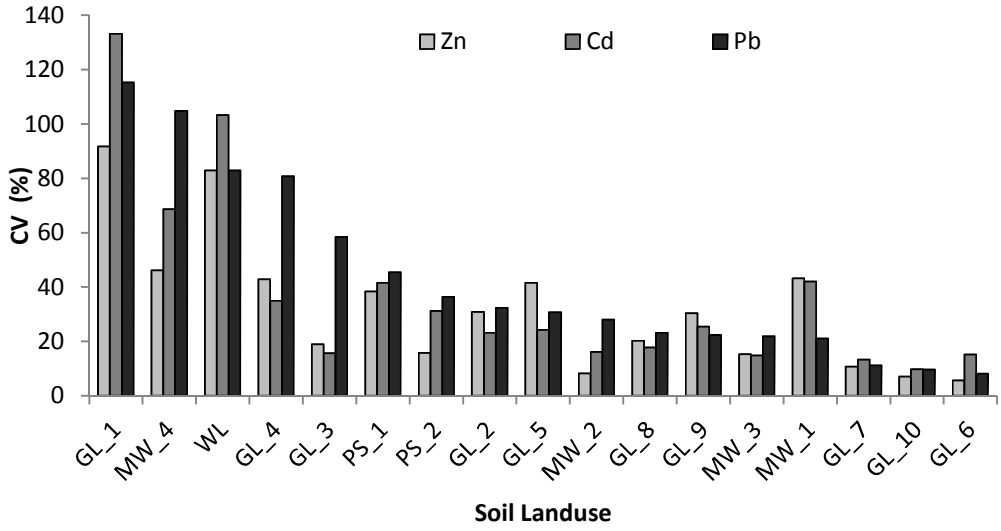

Fig. 4.7 Coefficients of variance (%CV) for all sampling locations (n = 17) represented as their dominant landuse. Data are arranged in descending order of %CV for Pb concentration. The X axis refers to the land-use for each sampling location: MW, Mine waste; WL, Woodland; PS, Peat soil and GL, Grassland.

4.4.2 Zinc, Cd and Pb E-value

4.4.2.1 The existence of 'non-labile colloidal' metal in the soil solution: E_{resin} *vs* E_{soln}

The Chelex-100 purification step used in the determination of E_{resin} (section 2.2.2), provides a proportion of 'non-labile colloidal' (NLC) metal in the soil solution. Fig. 4.8 shows that the two estimates of labile metal content (%E_{resin} and %E_{soln}) were highly correlated; r = 0.998, 0.987 and 0.982 ($P < 0.001$) for Zn, Cd and Pb respectively. The slopes from the regression equations based on the whole dataset (Zn-E_{soln} = 1.0257 Zn-E_{resin}, Cd-E_{soln} = 1.0226 Cd-E_{resin} and Pb-E_{soln} = 1.0189 Pb-E_{resin}) indicate that values of E_{soln} were, on average, 2.57%, 2.26% and 1.89% higher than those of E_{resin} for Zn, Cd and Pb respectively. Although this represents very good agreement between E_{soln} and E_{resin} there was a significant difference between the two estimates for all three metals ($P <$

0.001). The largest average difference between E_{soln} and E_{resin} values was found for Pb, followed by Cd, in all soil samples.

Fig. 4.9 shows the ratio of $\%E_{soln}$: $\%E_{resin}$ values as a function of soil pH and the total labile pool of Zn, Cd and Pb ($\%E_{resin}$). The deviation of E_{soln} from E_{resin} values was generally greater with increased soil pH for Cd and Pb and the slopes of the regression equations between E_{soln} and E_{resin} values for soils with pH ≥ 5.0 were greater than those measured for the whole dataset for Cd and Pb; Cd-E_{soln} = 1.0473 Cd-E_{resin} and Pb-E_{soln} = 1.0535 Pb-E_{resin}. This finding is in agreement with the observation that NLC metal is more mobile at high soil pH (Kaplan et al., 1996, Oste et al., 2002, Lombi et al., 2003). Furthermore, the presence of secondary carbonate minerals is likely to promote chemi-sorption processes, involving co-precipitation of non-labile metal pool on carbonate colloid surfaces. At low soil pH (≤ 3.0), the average ratios of E_{soln} : E_{resin} approached a value of 1.0 for Cd and Pb (Cd = 1.0149 and Pb = 1.0098). Ratios of E_{soln} : E_{resin} for Pb were larger and more scattered at low levels of metal lability (small E_{resin} values; Fig. 4.11). These trends are expected because as the lability of the metal increases there is less scope for a substantial effect from NLC metal and so E_{soln} gradually approaches the value of E_{resin}; in the extreme case of 100% lability, E_{soln} would have to equal E_{resin}. The soils with low metal lability were mainly contaminated with minespoil with relatively high soil pH values, suggesting a role for colloidal carbonate, or possibly Fe oxide, particles in the soil solution (Lombi et al., 2003).

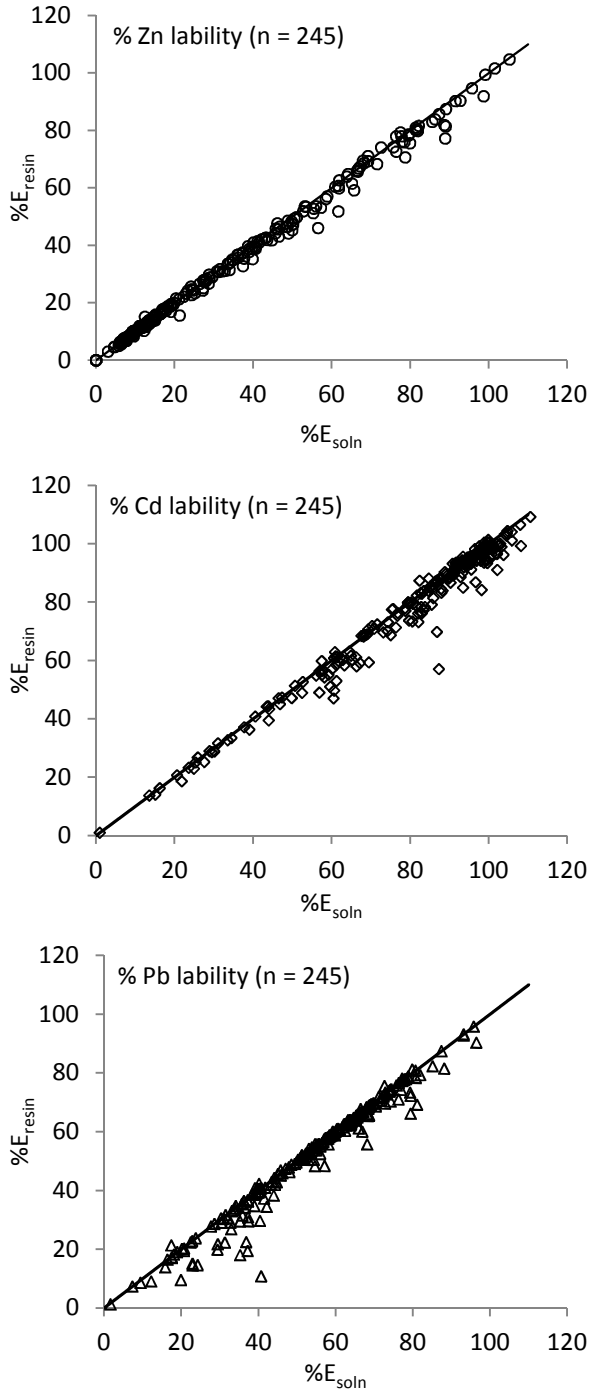

Fig. 4.8 Comparison of the isotopically exchangeable pool of Zn (○), Cd (◇) and Pb (△) measured as E_{soln} and as E_{resin} to demonstrate the occurrence of non-labile colloidal metal (n = 246). The average standard error (% total metal content) calculated for metal measured in the solution and resin phases were 1.49 and 1.10 % for Zn, 3.17 and 2.84 % for Cd and 1.87 and 1.78 % for Pb respectively. The solid line represents the 1 : 1 line.

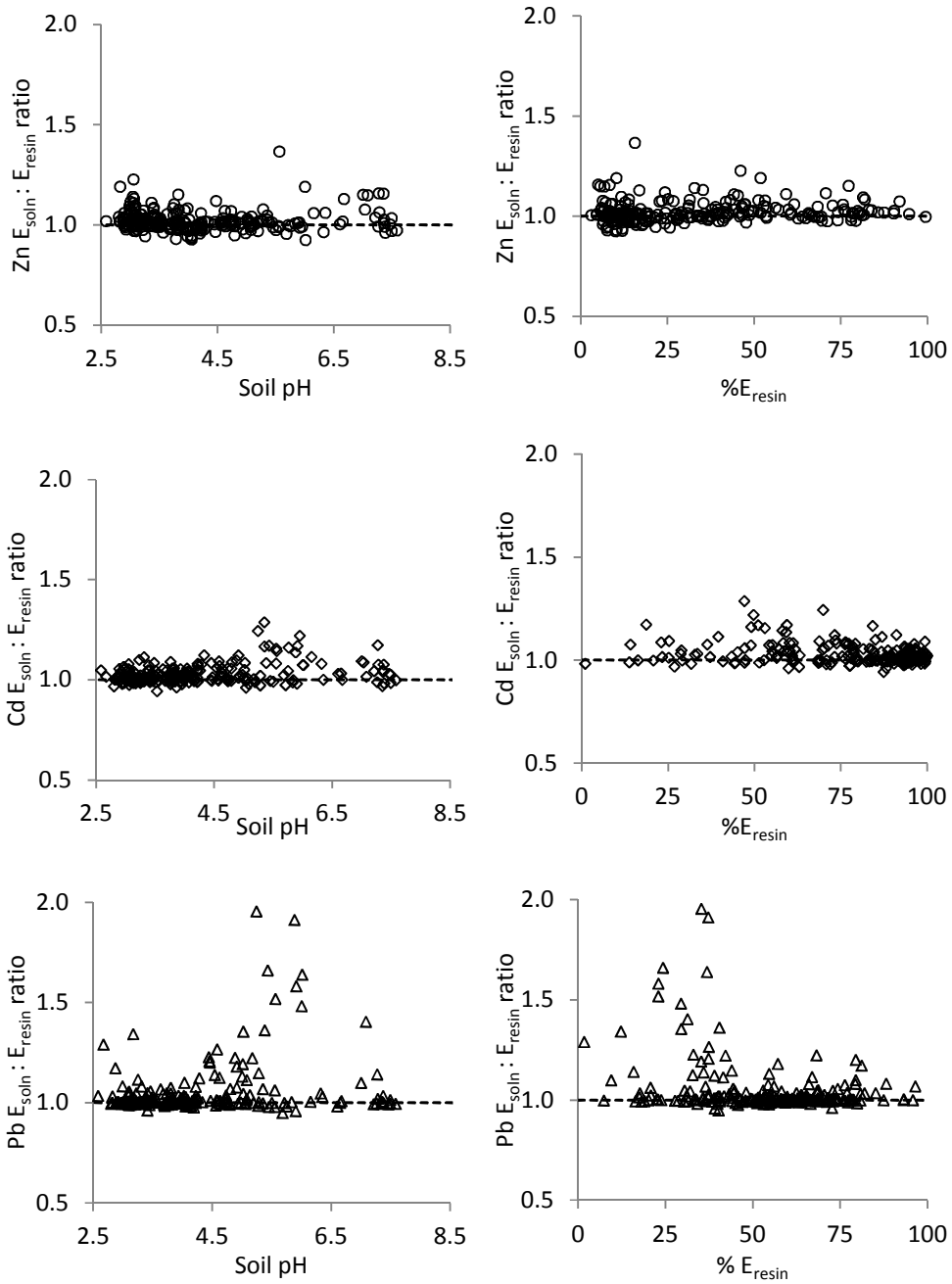

Fig. 4.9 The ratio of E_{soln} : E_{resin} as a function of soil pH and % metal lability (%E_{resin}) for Zn (○), Cd (◇) and Pb (△).

The nature of NLC metal can be influenced by soil physico-chemical properties and by the chemistry of the soil phase. To investigate the origins of NLC metal, correlation analyses were undertaken between the E_{soln} : E_{resin} ratio and soil characteristics that might be involved in the metal fixation process. Table 4.4 shows correlation coefficients (r) between the ratio of E_{soln} : E_{resin} and Al, Fe, Mn and DOC concentrations and pH values measured in the filtered (0.2 μm) soil

solutions (0.01M $Ca(NO_3)_2$). E-value ratios for Cd were significantly correlated with concentrations of Al and Fe and pH; ratios for Pb were correlated with concentrations of Al and DOC and pH (Table 4.4).

Table 4.4 Pearson correlation coefficients (r) between the ratio of E_{soln} : E_{resin} and pH and concentrations of Al, Fe, Mn in soil and DOC measured in the filtered (< 0.20 µm) soil solutions (0.01M $Ca(NO_3)_2$) (n = 246); P-values are in brackets.

	Zn	*Cd*	*Pb*
Al	0.065 (0.323)	**-0.263 (0.000)**	**-0.179 (0.006)**
Fe	-0.026 (0.692)	**-0.147 (0.024)**	-0.101 (0.120)
Mn	**-0.136 (0.036)**	-0.017 (0.791)	-0.024 (0.718)
DOC	0.058 (0.376)	-0.079 (0.225)	**-0.151 (0.020)**
pH	0.027 (0.683)	**0.302 (0.000)**	**0.244 (0.000)**

*Bold figures represent significant r values.

The nano-particulate phase of soils mainly consists of alumina-silicate clay particles, oxides of Fe and Mn, and organic colloids (Tack, 2010). The poor correlation results within the whole dataset did not provide a mechanistic explanation to characterize the type of any colloids which may be holding NLC metal. This is because the vast majority of the E_{soln} : E_{resin} ratios were close to a 1:1 ratio. Therefore, the characteristics of soils with Pb E-value ratios higher than 1.40 (n = 8) were more closely examined (Fig. 4.9). The pH and %LOI values of these soils were 5.24-7.08 and 9.02-18.6% respectively and they were all collected from the upper catchment underlain by Carboniferous Limestone (Shepherd et al., 2009). This may suggest that NLC-Pb was occluded within suspended $CaCO_3$ particles (< 0.2 µm). However, the Pb E-value ratio for these eight soils was also found to be positively correlated with DOC (r=0.53; P=0.18), Al (r=0.62; P=0.10), Fe (r=0.35; P=0.40) and Mn (r=0.79; P=0.02; Fig. 4.10). Thus, the correlation results suggest a possible association between NLC-Pb and

suspended Mn-oxides (< 0.2 μm). It is also notable that the soil with the highest E_{soln} : E_{resin} ratio for Pb (~ 2.0) compared to other soils (Pb E-value ratios, Fig. 4.9) had a pH of 5.24 and DOC of 17.2 mg L^{-1} which may suggest an association between NLC-Pb and dissolved organic matter.

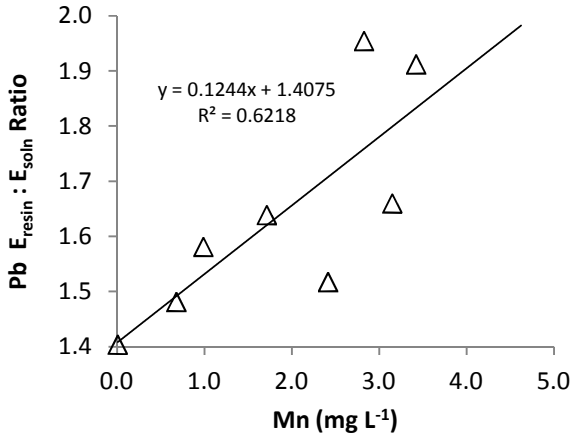

Fig. 4.10 Correlation of the ratio of Pb E-value, measured in solution and resin phase (E_{soln} : E_{resin}), and Mn concentration in the soil solution (Ca $(NO_3)_2$); n = 8.

4.4.2.2 Effect of soil properties on metal lability (E-value)

E-values (measured as E_{resin}) for all soil samples (n = 246) ranged from 1.47 to 2090 mg Zn kg^{-1}, 0.00285 to 17.2 mg Cd kg^{-1} and 1.19 to 4360 mg Pb kg^{-1}. Mean and (median) values (mg kg^{-1}) for Zn, Cd and Pb were 49.4 (12.8), 0.592 (0.262) and 497 (221) respectively. Expressed as a proportion (%) of total soil metal content, %E-values showed a very wide range across the soil samples collected from the Rookhope catchment, almost 1% - 100% for all three metals. However the range of %E-values also showed clear differences between the three metals; relative lability followed the sequence Cd > Pb > Zn (Fig. 4.11). Thus, Fig. 4.12 shows the frequency distribution of % E-values for Zn, Cd and Pb. As observed by Römkens et al.(2009) and Rodrigues et al. (2010), Cd was the most reactive metal. By contrast, Zn was considerably less labile than Cd which may either indicate its persistence in a primary sulphide mineral form (eg sphalerite) or secondary occlusion in minerals such as Fe oxides (Figures 4.11

and 4.12). Greater fixation of Zn than Cd in soil, following addition in solution, has been reported before by Smolders et al., (1999), Tye et al., (2003), Degryse et al., (2003a, and 2004) and Gabler et at. (2007) but there is little information on the relative stability of Zn and Cd primary ore minerals in soil. In fact, in the Rookhope soils subject to contamination with minespoil, it may be reasonable to assume that the Zn and Cd are initially closely associated and added to soil in isomorphically substituted sulphide minerals (Nakhone and Young, 1993). Therefore, the observation that Zn is considerable less labile than Cd (and Pb), appears to indicate contrasting geochemical pathways in soil. Thus the high correlation between ***total*** Zn and Cd concentration in these soils (r = 0.93; P = 0.000, Table 4.3), which suggests a continuing association in the pedogenic environment, may be misleading. Similarly, although the %E-values of Cd and Zn were significantly correlated (r = 0.50; P = 0.000), the slope was 0.57 indicating the lower lability of Zn.

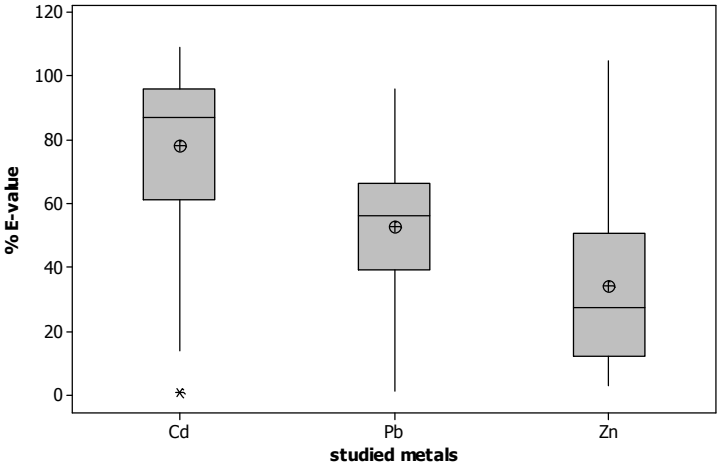

Fig. 4.11. % E-value ($\%E_{resin}$) for Cd, Pb and Zn in soils from the Rookhope catchment. The 'Box and Whisker' plot shows median (horizontal lines) and mean (symbols) for the three metals.

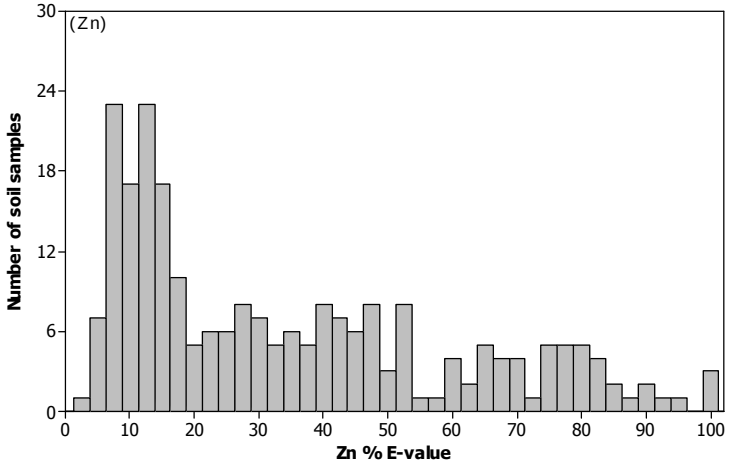

Fig. 4.12a. Frequency distributions of % E-values for Zn.

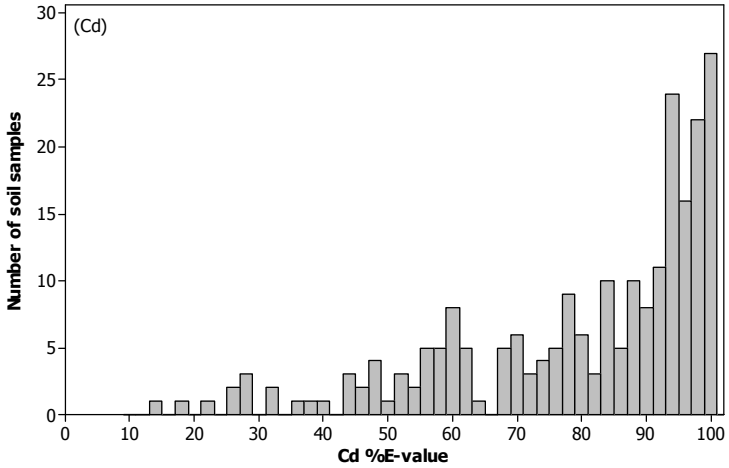

Fig. 4.12b. Frequency distributions of % E-values for Cd.

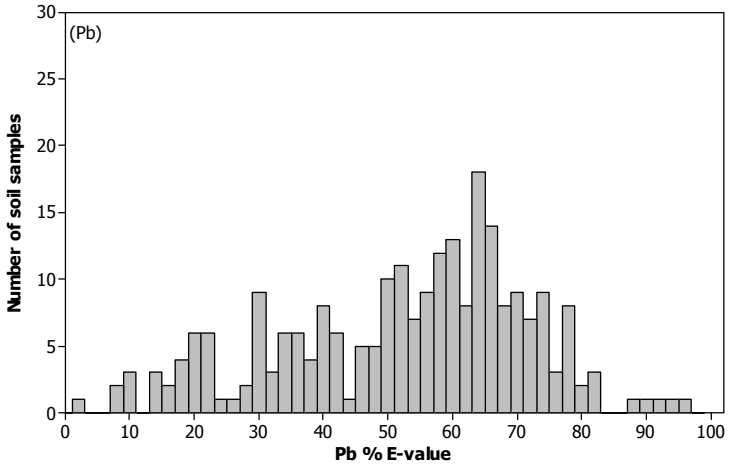

Fig. 4.12c. Frequency distributions of % E-values for Pb.

Effect of total metal content on lability

The soil organic matter content, pH and total metal concentration explained most of the variation in % E-value. Table 4.5 shows correlation coefficients (r) and P-values between %E-value and (i) soil organic matter (as % LOI), (ii) pH and (iii) total metal concentration of Zn, Cd and Pb. The results show a significant negative correlation ($P < 0.001$) between the total metal content and the % E-value for Zn (r=-0.28), Cd (-0.49) and Pb (-0.37). The poor, but significant, correlations probably reflect a combination of 'source characteristics' and soil properties within the full dataset (n = 246). The soils directly affected by 'minespoil' may retain primary minerals (such as sulphides and carbonates) and their large metal contents and calcareous nature will facilitate the formation of secondary minerals such as mixed carbonates.

Table 4.5 Pearson correlation coefficients (r) between the % E-value and pH, %LOI and total metal concentrations in soil for Zn, Cd and Pb (mg kg^{-1}); P-values are in brackets.

Soil variable	*% E-value*		
	Zn	Cd	Pb
Total Zn	-0.282 (0.000)	-0.565 (0.000)	-0.423 (0.000)
Total Cd	*-0.075 (0.253)*	-0.490 (0.000)	-0.349 (0.000)
Total Pb	-0.212 (0.001)	-0.534 (0.000)	-0.373 (0.000)
%LOI	0.848 (0.000)	0.386 (0.000)	0.496 (0.000)
pH	-0.545 (0.000)	-0.686 (0.000)	-0.751 (0.000)

In soils directly contaminated by minespoil and with a high pH to preserve the metal carbonate fraction and encourage fixation of metal ions released from sulphides, it is more likely to see a negative relationship between % lability (% E-value) and total metal content. A larger metal content would simply mean a larger proportion of metal in minespoil material and so a greater proportion of

non-labile and fixed metal. However, in acidic organic soils with a comparatively low metal content it is likely that the original input from smelters and minespoil dust has been completely dissolved and adsorbed by the soil. Therefore, the %lability is likely to be a function of how strongly the metal is held within the soil, mainly by humus and oxides. A smaller metal content is then more likely to be associated with the 'strongest' adsorption sites and further addition of metal will be associated with progressively weaker adsorption sites. Under those circumstances therefore, the expectation would be for a positive correlation between %lability and metal content. Therefore, there are two potentially conflicting trends in %lability and total soil metal content, depending on whether the soil metals are mainly (i) held on soil adsorption sites or (ii) associated with primary or secondary ore minerals. The overall relationship seen may result from a combination of (i) and (ii).

Within the dataset from Rookhope there is some evidence for such a binary trend in the relationship between %lability and soil metal content for Zn, Cd and Pb. Fig. 4.13 shows that the relationship between total metal concentration (mg kg^{-1}) and % E-values for Zn, Cd and Pb. The data were divided into two categories, based on total metal concentration, with small black symbols and large grey symbols representing low and high metal contents respectively. The divisions (X-axis) between the two categories were set to 225, 0.70 and 2000 mg kg^{-1} for Zn, Cd and Pb respectively. These rather arbitrary divisions were established by optimisation (R^2) of an exponential fit to data in the high metal concentration category for Zn, Cd and Pb.

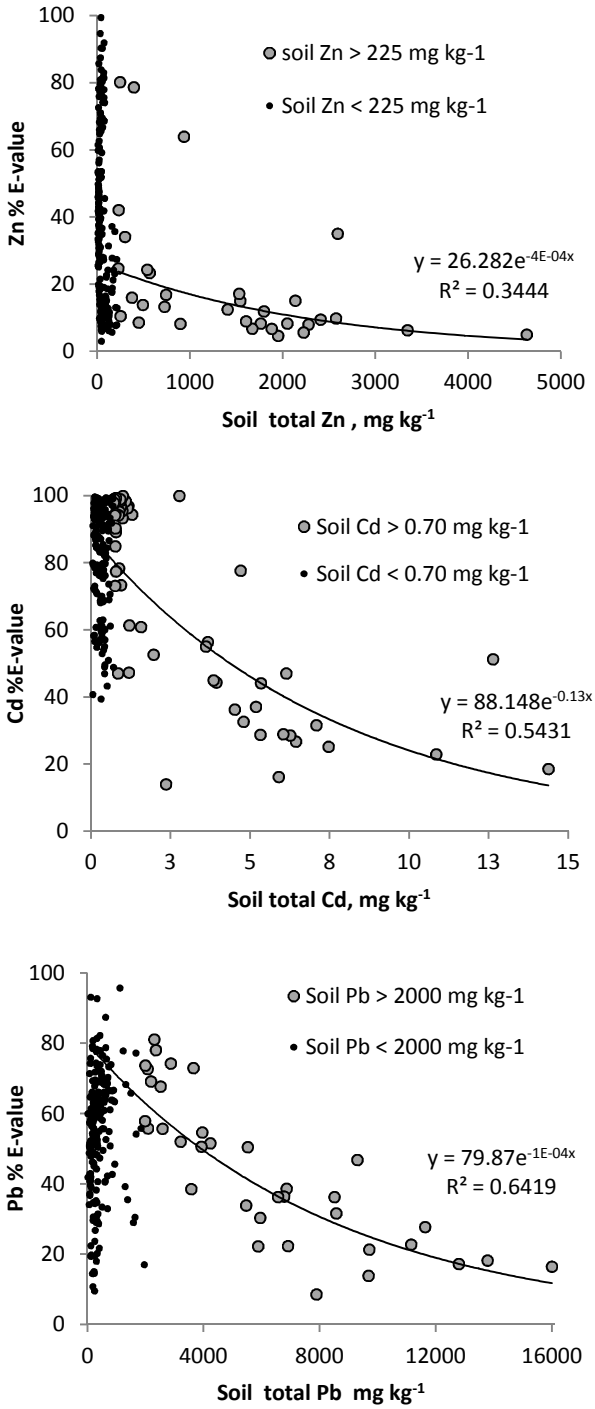

Fig. 4.13 Relationship between E-value (% of total) and soil total metal concentration (mg kg^{-1}) of Zn, Cd and Pb. The large grey and small black symbols represent the high and low total metal concentration categories, respectively; sold line represents an exponential fit to the high concentration category.

In general, there were two obvious trends for the three metals particularly in the case of Pb. The negative correlation coefficients (P <0.01) between the total metal concentration and % labile pool were greater for soils with 'large' metal concentrations,(***Zn > 225*** *mg* kg^{-1}; n = 33; r = -0.46, ***Cd > 0.7*** *mg* kg^{-1}; n = 55; r = -0.74 and ***Pb >2000*** *mg* kg^{-1}; n = 35; r = -0.82); compared to the full dataset for the three metals (Table 4.5). Most of these soils had relatively low organic matter content and high pH values. Under those circumstances, Zn, Cd and Pb ions are more likely to be adsorbed into inorganic substrates, such as carbonates and Fe & Mn oxides which, in contrast to humus, are capable of fixing the metals in a non-labile form as expected. In addition, soils with high pH and large metal contents are more likely to have residual primary ore minerals and therefore a greater proportion of non-labile and inert metal. By contrast, the reverse trend was apparent in the case of Pb in soils with low metal concentrations (< 2000 mg kg^{-1}) where a positive correlation (n = 199; P = 0.202) was found between % E-value and soil Pb content (r = 0.10). Although Zn and Cd % E-values showed still produced a negative correlation (P < 0.01) at low metal concentrations (***Zn < 225***; n = 199; r = -0.36 and ***Cd < 0.70;*** n = 177; r = -0.21) the values of r were much smaller than for the full dataset for Cd and Zn. The soils with low metal contents generally had low pH values and relatively high organic matter contents. The acidic soil samples with low metal contents were typically from upland areas of the catchment, relatively far from the minespoil and more likely to have received low level metal deposition as dust or smelter output. This, in general, would result in a larger labile metal fraction than in the minespoil soils following weathering under acid conditions (Egli et al., 2010) with metal ions held mainly by humus and Fe/Mn oxides.

There is therefore a binary trend in %E-value with total soil metal content within the Rookhope dataset which is explainable as a combination of soil adsorption characteristics and the presence of metal-enriched primary minerals. The second trend, at higher metal concentrations is clearly strongest but in the case of Pb,

especially, there was also evidence of a primary trend associated with progressively weaker adsorption strength as metal content increased.

Effect of soil pH and soil organic matter

Many studies have demonstrated the important of pH (McBride et al., 2006, Bonten et al., 2008) and organic matter (Tipping et al., 2003, Rodrigues et al., 2010) on metal lability in soils. As expected, the results indicated that metal %lability increased with decreasing soil pH ($P < 0.001$) for Zn ($r = -0.53$), Cd ($r = -0.57$) and Pb ($r = -0.66$) (Figures 4.14 and 4.15). The same trend was observed by Tye et al., (2003) and Degryse et al., (2004). Many reports have shown that soil pH has the greatest effect on metal lability (Martinez and Motto, 2000, Schulin et al., 2010). The underlying mechanisms for this trend may partly lie in stronger bonding between the metal ions and oxygen atoms in the soil solid phase as pH increases (Crout et al., 2006 and the references therein). Increasing soil pH presents both lower H^+ competition for available oxygen atoms and more favourable electrostatic potentials for metal sorption. Other reasons for lower lability at higher pH values may be a change in metal fractionation, from humus-bound to adsorption on Fe/Mn oxides for example and, in the particular case of minespoil-contaminated soils, the increasing presence of primary ore-body minerals.

Humus is an important soil component that may constitute the major reservoir of labile metal (Ottosen et al., 2009). Positive significant correlations ($P < 0.001$) were observed for % LOI and % E-value for Zn ($r = 0.84$), Cd (0.39) and Pb (0.49) (Fig. 4.15). Sarret et al. (2004) studied Zn speciation in contaminated soils by mining activities (Nord, France) using EXAFS and isotopic dilution techniques. Their results showed that labile Zn ranged from 54 to 92% and the main form of labile Zn was organic (~ 45%), while only ~ 15% of the total soil Zn was found as primary minerals. The poorer correlations for %LOI and % E-value for Cd and Pb may have been influenced by the very low soil pH in some

soils (e.g. pH 2.7) possibly due to the presence and oxidation of pyrite (FeS) which would produce high levels of lability in soils quite low in organic matter. There was quite a poor correlation between soil pH and % LOI ($r = -0.42$; $P < 0.001$).

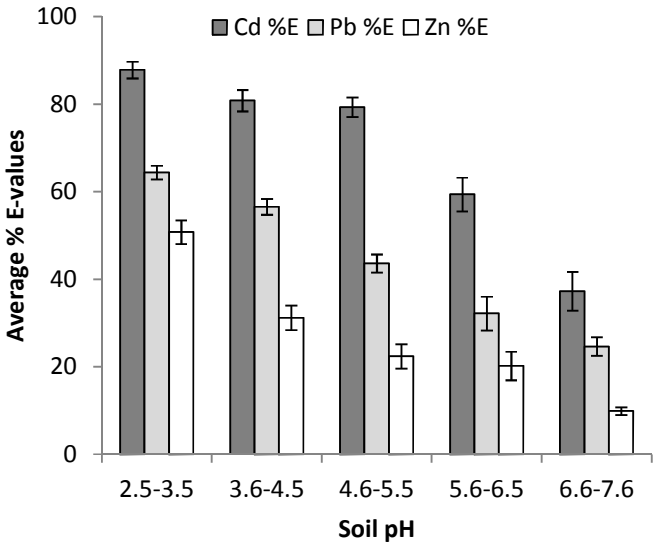

Fig. 4.14 Average metal lability for Zn, Cd and Pb grouped into soil pH classes. The error bars show the standard errors among soils with the same pH classes.

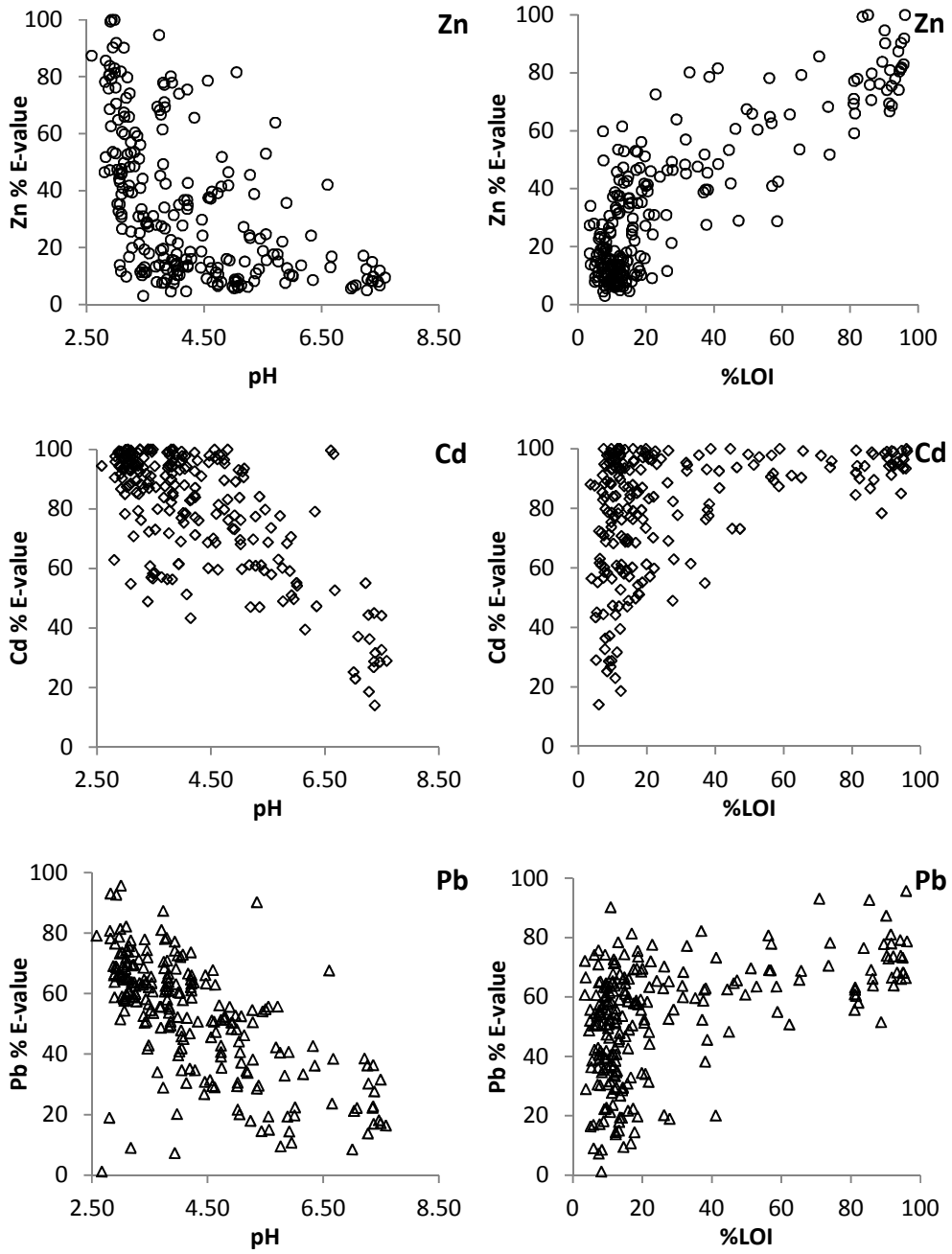

Fig. 4.15 Relationship between E-value (% of total) and soil pH and % LOI for Zn (O), Cd (◇) and Pb (△)

Multiple regression model to predict metal lability

According to the correlation analysis between metal lability and soil properties (pH, %LOI and total metal content), multiple regression models were used to predict the exchangeable metal pool of Zn, Cd and Pb (Fig. 4.16) as illustrated by equation 4.1:

$$\mathrm{M_L} = a + b\,\mathrm{pH} + c\,\mathrm{M_T} + d\,\mathrm{OM} \qquad \text{eq 4.1}$$

where regression coefficients (a, b, c and d), can be positive or negative (see Table 4.6), and M_L and M_T are the isotopically exchangeable metal fraction (% E-value) and total metal concentration (mg kg^{-1}), respectively, of Zn, Cd and Pb while OM refers to soil organic matter content measured as % LOI. Between eleven to thirteen soil samples (from 246 samples) were omitted from the prediction model for Zn, Cd and Pb as atypical outliers. In general, the soils that were excluded from the model had very low metal lability (e.g. in case of Cd; less than 0.08%) and/or different sources of metals contaminants such as vehicle tire debris, observed during sampling (e.g. Zn and Pb).

Based on the previous observation of a binary trend between metal lability and total metal content for Zn, Cd and Pb, two multiple regression models were simultaneously applied to the soils, allocated on the basis of total metal concentration. The dividing points for the two groups of data were 225, 0.70 and 2000 mg kg^{-1} for Zn, Cd and Pb respectively. The overall goodness fit for all measured and modelled metal lability was determined based on R^2 and the residual standard deviation (RSD) (equation 4.2):

$$\mathrm{RSD} = \sqrt{\frac{1}{\mathrm{n\text{-}c}} \sum_{i=1}^{n} (M_i\text{-}P_i)^2} \qquad \text{(eq. 4.2)}$$

Where M_i and P_i are the measured and predicted values of % lability using equation 4.1, n is the number of the observed values and c is the number of fitted coefficients in equation 4.1 (typically 8).

Approximately 80%, 53% and 66% of the variability in the isotopically exchangeable pool of Zn, Cd and Pb, respectively, were explained by the variation in soil pH, %LOI and total metal content (Table 4.6); RSD values (% total metal content) were a little over 10%. Fig. 4.16 shows that the predicted labile metal fractions were in reasonable agreement with the measured values particularly for Zn and Pb (R^2 = 0.80 and 0.66; RSD = 11.5 and 10.6,

respectively) while the lowest model performance was observed with Cd (R^2 = 0.53 and RSD = 14.2).

Previous authors have mainly focussed on prediction of metal solubility. For example, Gray and McLaren (2006) used basic soil properties (pH, total carbon and total metal content) to predict metal *solubility* in a wide range of soils. They found that for Cd only 50% of the variation could be predicted by pH, total carbon and total Cd content.

Table 4.6 Linear regression coefficients (a, b, c and d), RSD and R^2 values of individual equations for prediction model of % metal lability; the multiple regression models were applied to two subsets of the full dataset simultaneously based on the total metal concentration, mg kg^{-1} (see text for more details); P-values are in brackets.

	Zn		**Cd**		**Pb**	
Total metal (mg kg^{-1})	**< 225 (n = 199)**	**> 225 (n = 33)**	**< 0.7 (n = 177)**	**> 0.7 (n = 55)**	**< 2000 (n = 199)**	**> 2000 (n= 35)**
a (intercept)	39.6 (0.000)	15.5 (0.129)	108 (0.000)	110 (0.000)	102 (0.000)	89.7 (0.000)
b (pH)	-5.06 (0.000)	-1.36 (0.39)	-5.63 (0.000)	-8.48 (0.001)	-13.1 (0.000)	-5.61 (0.004)
c (Total metal)	-0.0652 (0.003)	-0.00562 (0.007)	-24.8 (0.007)	-1.72 (0.023)	0.00663 (0.005)	-0.00254 (0.000)
d (% LOI)	0.674 (0.000)	1.803 (0.000)	0.201 (0.002)	0.176 (0.103)	0.838 (0.013)	0.0966 (0.181)
RSD (% metal content)	11.5		14.2		10.6	
R^2	0.801		0.533		0.664	

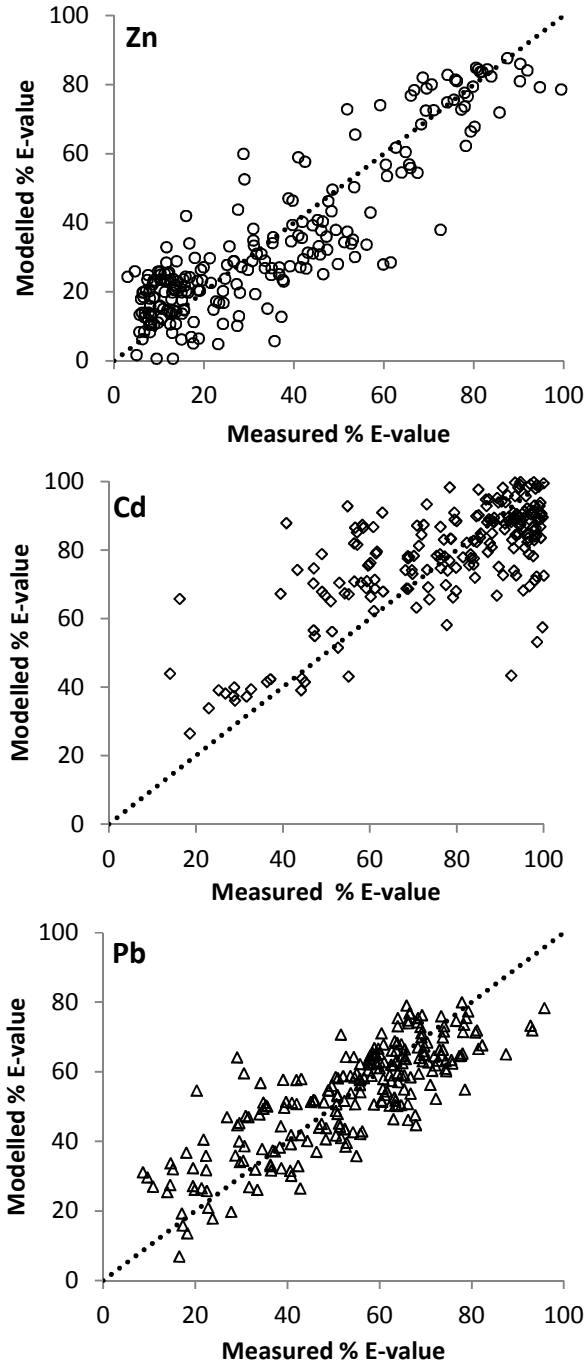

Fig. 4.16 Predicting Zn (O), Cd (◇) and Pb (△) lability (% E-value) from pH, total metal concentration and % LOI (equation 4.1); broken lines represent the 1 : 1 relation.

Although secondary minerals such as Fe and Mn oxides are known to be important sorbents for a range of metals (Trivedi and Axe, 2000), the inclusion of this additional parameter (amorphous Fe and Mn oxide content) within the

regression equation (4.2) provided no apparent improvement in model RSD values over that of equation 4.1 ; RSD (R^2) = 11.3 (0.81), 14.8 (0.58) and 10.5 (0.67) for Zn, Cd and Pb respectively.

The effects of the basic soil properties in the empirical models for predicting metal lability were generally in accordance with processes expected to occur within the wide range of soil samples collected from Rookhope catchment (Table 4.5). Soil pH had a significant effect on prediction of % E-value at both low and high levels of metals for all three metals with the exception of large soil Zn contents (Table 4.6). The majority of soil samples were present at 'low' metal levels (Zn <225 mg kg^{-1}; n = 199). Although soil pH is important in governing strength of adsorption (Gabler et al., 2007), which may influence lability, the significance of soil pH in the empirical model may also arise from a correlation with other soil characteristics (e.g. total metal concentration and soil organic matter content). Soil organic matter content showed a significant influence on % E-values under both low and high Zn contents ($P < 0.001$) but only at low concentrations of Cd and Pb ($P < 0.05$). However, humus content had no significant effect ($P > 0.05$) at high Cd and Pb loadings where association with carbonates and possibly Fe and Mn oxides may have been more important. Total metal content had a significant effect on prediction of % E-value at both low and high levels of metal content ($p < 0.05$) for the three metals. The regression coefficients for total metal content at high metal concentrations were higher than those at low metal contents by one order of magnitude for Zn and Cd.

It is possible that such multiple regression models used to predict metal lability could be improved by including more variables such as the cation exchange capacity (CEC) or free ion concentration in the soil solution phase. However, from a pragmatic point of view, it is perhaps preferable that prediction should involve only fairly simple soil properties (pH, % OC and total metal content) that are also used within geochemical speciation models. From the multiple

regression analysis in the current study, it was found that the soil total metal concentration had the most significant effect on prediction metal lability (%), followed by soil pH. Soil organic matter was most significant at low metal concentrations. Finally, it should be acknowledged that part of the regression between % E-value and total soil metal content arises from the inclusion of total soil metal content in both variables. This means that even a completely random distribution of E-value would produce some degree of correlation between % E-value and total metal content.

4.4.3 Predicting soluble metal concentrations from concentration of isotopically exchangeable metal using WHAM VI

Metal concentrations measured in the 0.01 M $Ca(NO_3)_2$ electrolyte used to determine E-values can be used as a proxy for pore-water concentrations. Degryse et al. (2003a, 2011) used Zn and Cd concentration extracted by 0.01 M $CaCl_2$ to predict their solubility in the soil solution phase. Therefore, the soil-electrolyte suspensions used in the measurement of isotopically exchangeable metal can also be used to test the ability of a geochemical model to predict solubility. The E-values for Cd, Zn and Pb were therefore matched with measurements of major and minor cations, anions, DOC and pH made in the solution phase of the electrolyte suspensions, and Fe/Mn oxide content and LOI values for the individual soils, to provide input data for WHAM(VI) as described in section 2.4.

Fig. 4.17 shows observed versus predicted values of metal solubility using WHAM(VI) parameterized with either metal E-values or total content of Zn, Cd and Pb; Table 4.7 shows the relative predictive power of the two approaches.

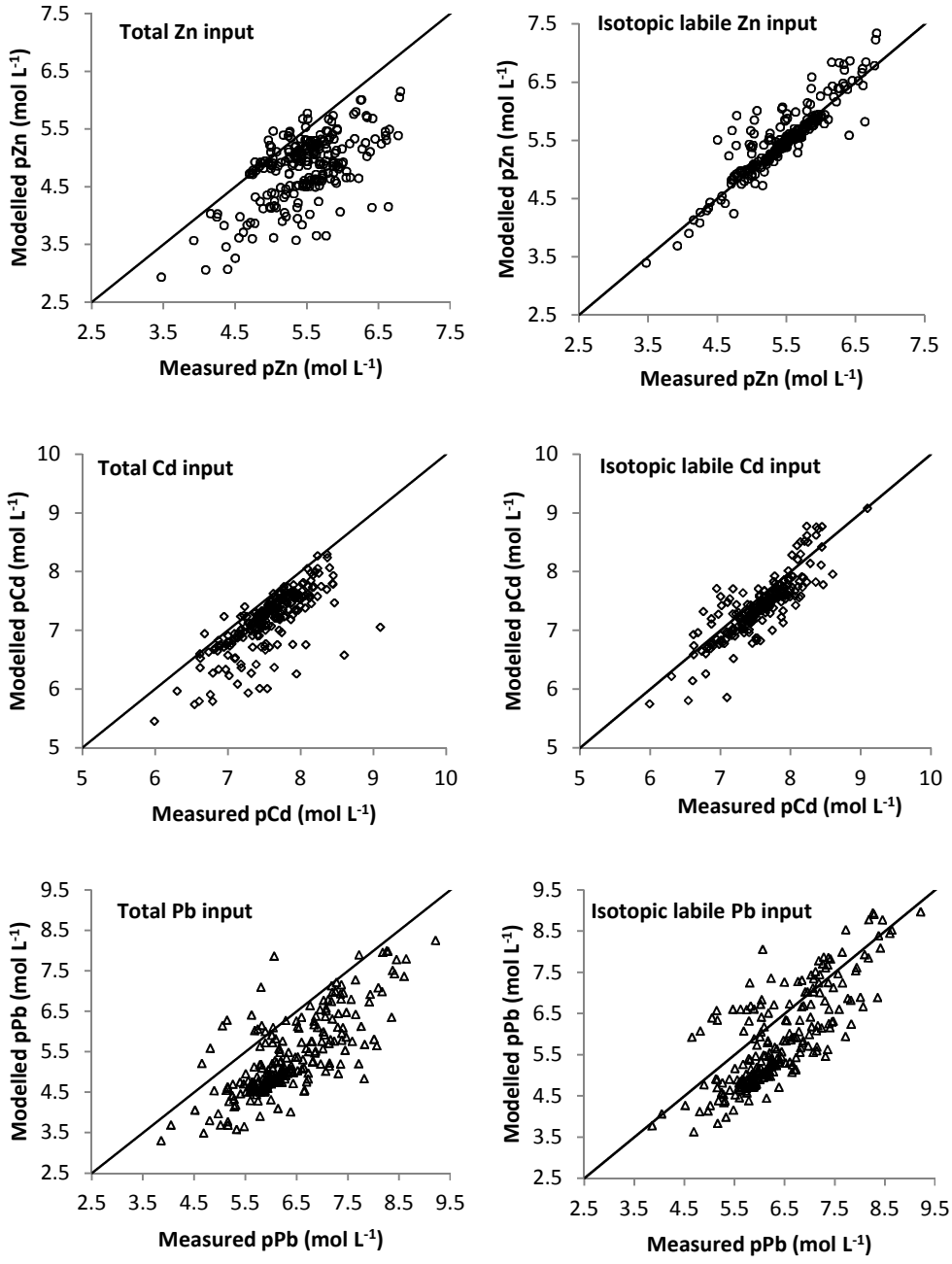

Fig. 4.17. Predicted versus observed soluble metal concentrations (mol L^{-1}, -$\log_{10}$ scale) in a 0.01 M $Ca(NO_3)_2$ soil extract (n=241). Predictions were made with WHAM/Model VI, parameterized by either total metal or isotopic labile metal pools of Zn, Cd and Pb. The sold lines represent the 1:1 lines.

Table 4.7. Values of R^2, RSD, *median* predicted-to-measured ratio (P/M) and *mean* deviation on a log scale (ΔpM_{soln}) for the simulation of soluble Zn, Cd and Pb concentrations (in a 10 mM $Ca(NO_3)_2$ extract) in soil suspensions (n = 241 for each metal). Predictions were made using WHAM(VI) with either the metal E-value or the total soil metal concentration as input.

	Zn		*Cd*		*Pb*	
	Total	E-value	Total	E-value	Total	E-value
R^2	0.35	0.81	0.57	0.72	0.58	0.61
RSD	0.80	0.46	0.49	0.30	1.18	0.90
Median P/M ratio	3.70	1.02	1.92	1.49	12.5	6.48
Mean ΔpM_{soln}*	-0.63	0.08	-0.37	-0.15	-0.99	-0.56

* ΔpM_{soln} = pM_{soln} predicted – pM_{soln} measured

The results clearly demonstrate that predictions based on total metal content are less successful than those based on E-values (Fig. 4.17 and Table 4.7), as suggested by Buekers et al. (2008b). Using total metal concentration as input to WHAM(VI), generally overestimated the total soluble metal concentrations for the three metals particularly in the case of Pb (median P/M ratio = 12.5, see Table 4.7). The R^2 values for measured vs modelled values were also poor: Zn = 0.35, Cd = 0.57 Pb = 0.58 with RSD ($-\log_{10}M_{soln}$) values of 0.80, 0.49 and 1.18 respectively. Concentrations of soluble metals were reasonably well predicted using E-values as input variable to WHAM(VI), with the exception of Pb. Nevertheless, the prediction of Pb solubility also improved by using isotopically exchangeable Pb as an input to WHAM(VI).

Better predictions for Zn (R^2 = 0.97) and Cd (0.95) were obtained by confining the dataset to acidic soils with pH values less than pH 3.5 where the solubility is greater and adsorption is more likely to be on humus rather than Fe oxides (Fig. 4.18).

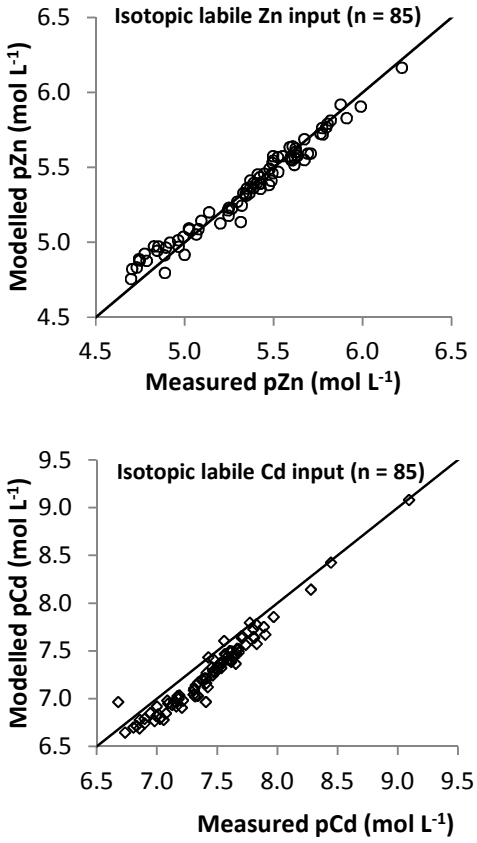

Fig. 4.18. Predicted versus observed soluble metal concentrations (mol L^{-1}; -$\log_{10}$ scale) in a 0.01 M $Ca(NO_3)_2$ soil extract (n=85; all pH values < 3.5). Predictions were made with WHAM(VI), parameterized by isotopically exchangeable Zn and Cd. The sold lines represent the 1:1 line.

Output from the WHAM(VI) model applied to 'whole soils' also includes (predicted) metal fractionation in the solid phase. Predicted metal association with (solid) humic acid, Fe and Mn oxides or dissolved in the solution phase (free ions and all complexes, including those with fulvic acid) are presented as a function of pH in Fig. 4.19.

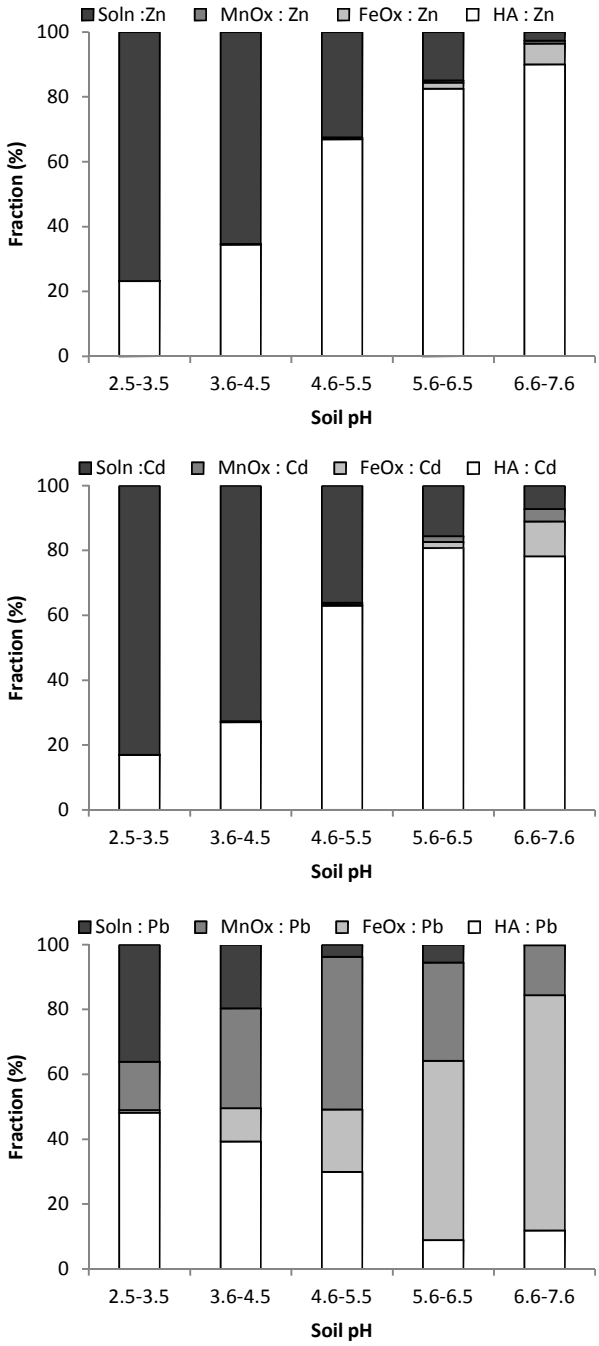

Fig. 4.19 Average metal distribution on Humic Acid (HA), Fe and Mn oxyhydroxides (FeOx and MnOx) and in the solution phase (soln) for Zn, Cd and Pb in the Rookhope soils (n = 241) grouped by pH class, as predicted by WHAM parameterized with isotopically exchangeable metal.

The results confirm that most of the Zn and Cd pool is present in the solution phase at low soil pH (< 4.0). Therefore, the median ratio of predicted to measured values (P/M ratio; Table 4.7) was very close to 1.0 for Zn (1.02) and Cd (1.49). Although using Cd E-value as input to WHAM(VI) improved the prediction of solubility, the P/M ratio derived using total metal concentration was quite close to results using E-value (Table 4.7). This is because the majority of Cd had E-values closer to 100% of the total metal content (Fig. 4.12) compared to Zn and Pb.

Fig. 4.19 also shows that the fraction of Zn and Cd adsorbed to iron oxyhydroxides and humic acids increased with increasing pH but the amount of the metal adsorbed to humic acids was substantially greater than that adsorbed to oxides. Weng et al. (2004) showed a contrary trend but the soils used in their study had much lower organic matter contents (n = 3, %LOI: 4-8%) than the Rookhope catchement soils used in the current study (n = 246, %LOI: 4-96%). At pH > 5 humus was the most important adsorbent for both Zn and Cd (> 80% adsorbed to humic acids) (Fig. 4.19).

Predicted fractionation of Pb was more complex (Fig. 4.19) with both soluble and humic-bound Pb declining as pH rose with a gradual increase in the fraction bound to Fe oxides; Pb bound to MnO_2 appeared to pass through a maximum in the pH range 4.6 – 5.5. The model generally overestimated the total soluble Pb but with a high degree of scatter around the 1:1 line (Table 4.7 and Fig. 4.17). Other studies have shown more consistent overestimation of Pb solubility than in the current study (Weng et al., 2002, Schroder et al., 2005). This again may reflect the advantage of using the isotopically exchangeable pool of Pb as an input to the WHAM(VI) model in comparison to traditional chemical extractions – especially in calcareous soils where acid extractions probably produce over-estimates of the labile Pb pool (Chapter 3). However, it is usually found that

solution speciation of Pb is reasonably described with geochemical models such as WHAM(VI) (Weng et al., 2002, Bonten et al., 2008).

There are various possible explanations for the failure of the model to predict Pb solubility. For example, the extraction methods used to determine the labile Pb fraction may release some non-labile Pb; however, this potential failure was addressed in the current study as isotopically exchangeable Pb was used. Other explanations include a failure to account for (i) synergistic effects associated with adsorption on mixed oxide-humic surfaces (Heidmann et al., 2005), (ii) other important adsorbents that are not incorporated in the current model, (iii) the formation of ternary complexes such as Fe-PO_4-Pb on Fe oxides (Weesner and Bleam, 1998, Linde et al., 2007) and (iv) the existence of highly specific sites on Fe oxides with much greater intrinsic stability constants than used in the general surface complexation model (Buekers et al., 2008b). Given the Pb fractionation pattern shown in Fig. 4.19 and the greater spread of results at low levels of Pb solubility and high pH values (Fig. 4.17) it seems likely that the main failing in predicting Pb solubility in whole soils lies with the description of binding to Fe oxides.

Fig. 4.20 shows the ratio of predicted to measured concentration of soluble Pb (mol L^{-1}) as a function of soil organic matter and pH. In general, modelled values overestimated the Pb concentration (P/M ratio > 1) and there was no consistent trend either with pH or with humus content (% LOI).

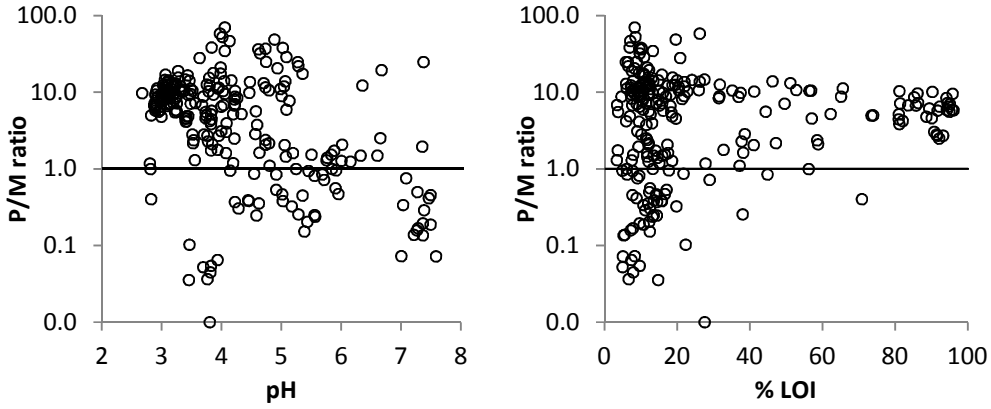

Fig. 4.20 Ratio of predicted:measured concentrations of Pb concentration (mol L^{-1}) in solution (log_{10} scale) as a function of soil organic matter (%LOI) or soil pH. Solid lines indicate a ratio of one.

The relative error between predicted and measured Pb concentration was calculated by equation 4.3:

$$RE = \frac{\sqrt{(P_{Pb} - M_{Pb})^2}}{M_{Pb}} \qquad \text{Eq. 4.3}$$

Where RE is relative error, P_{Pb} and M_{Pb} are the predicted and measured values of Pb_{soln}. In Fig. 4.21 the RE is shown as a function of the proportion of Pb associated with humic acid (HA:Pb) and Fe oxides (FeOx:Pb) as predicted by WHAM(VI) (Fig. 4.21).

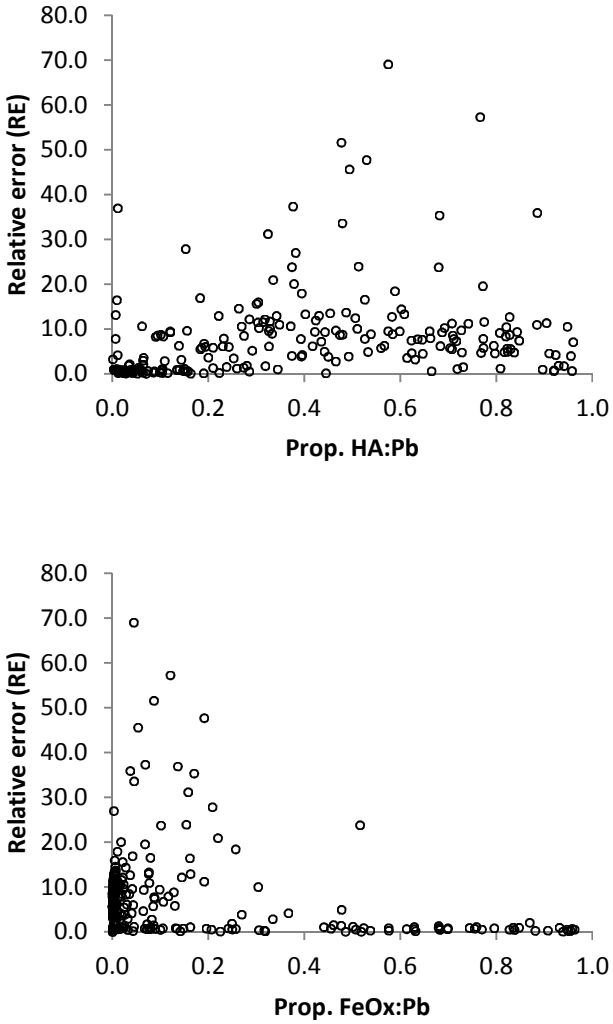

Fig. 4.21 Relative Error (RE) between modeled and measured Pb solubility as a function of the proportion of Pb associated with humic acid (HA-Pb) and Fe oxides (FeOx-Pb) as predicted by WHAM(VI) parameterized by Isotopically exchangeable metal pool.

Contrary to expectation, RE values increased with increasing HA-Pb suggesting that poor prediction may be due to the unexpectedly strong binding to soil organic matter. This suggestion is in agreement with Linde et al. (2007). They demonstrated that the failure of Pb solubility model might be explained by stronger complexation with soil organic matter and Fe oxyhydroxides. Moreover, Fig. 4.19 shows the % FeOX-Pb increases with increasing pH while % HA-Pb decreases with increasing pH. Again, perhaps against expectation, it was found

that RE decreased with increasing FeOX-Pb (Fig. 4.21), suggesting that the description of Pb binding to Fe oxides in WHAM(VI) is quite soundly based and parameterised.

Another possible explanation for poor prediction of Pb solubility is unaccounted for complexation with Mn oxides. Significant association with Mn oxides was predicted in soils with pH values from 4.6 to 5.5 (47%) (Fig. 4.19). Several publications have concluded that Pb is strongly complexed by Mn oxides (Lofts and Tipping, 1998, Trivedi and Axe, 2000, Cances et al., 2003).

It is therefore difficult to identify a single distinct reason to explain the poorer Pb fits. However, various improvements to the model, and to the input data, may help. Since bonding to humic acid does seem to be important and subject to error (Fig. 4.21) optimizing the 'active soil organic matter' content as an input to WHAM(VI) could resolve part of the model deviation. In addition to the issues raised earlier, better methods for determining active oxide content and greater allowance for heterogeneity in bonding strength to oxide surfaces (especially Mn oxides or perhaps hydroxy-interlayered clays) within the model may also contribute to better prediction. Nevertheless, although the prediction of soluble Pb concentration predicted by WHAM(VI) from Pb E-values shows substantial deviation from the measured values, the fit to the data in the current study is probably better than of those currently published in the literature and predicted using chemical extraction Pb (Weng et al., 2002, Tipping et al., 2003, Schroder et al., 2005, MacDonald and Hendershot, 2006).

5. CHAPTER 5: CHANGES IN METAL SOLUBILITY AND LABILITY UNDER REDOX CONDITIONS

5.1 Overview

Redox potential (Eh, mV) is one of the key factors governing the lability and bioavailability of some metals and metalloids in soils (Borch et al., 2010). The strength of oxidizing or reducing conditions is expressed as Eh and typically measured using as the potential difference between a platinum and reference electrode (Ponnamperuma, 1972). The relationship between soil Eh value and the sequence of redox reactions with progressive reduction are presented in Fig. 5.1.

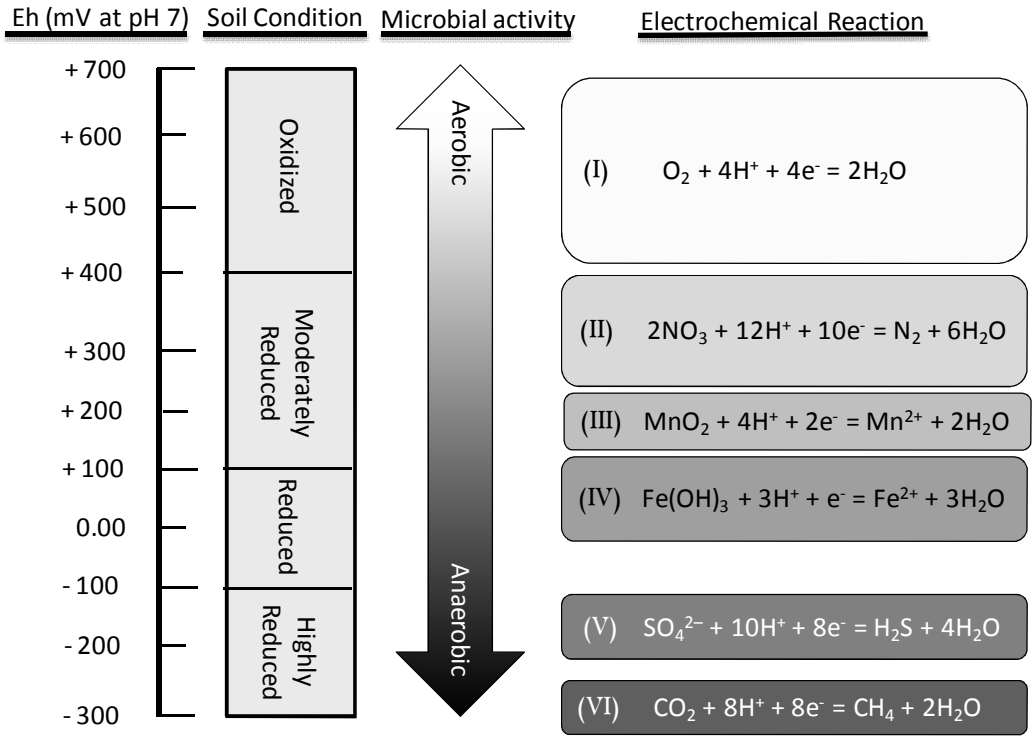

Fig. 5.1 Schematic presentation of the relationship between soil Eh value and the prevailing electrochemical reactions which buffer (or 'poise') redox potential in soils. This diagram was developed from published information and concepts in the literature (Ponnamperuma, 1972, Zhi-Guang, 1985, Masscheleyn et al., 1993, Patrick, 1996, Ramesh and DeLaune, 2008, Florido et al., 2011).

In soils that experience seasonal fluctuations in moisture content Eh values can be highly variable. Well drained soil is normally aerated and hence oxidizing (Eh = +400 to +700 mV) (Patrick, 1996), while flooded soil is subject to reducing conditions, ranging from moderately reduced (Eh = +100 to +400) (Masscheleyn et al., 1993), reduced (Eh = -100 to +100) to highly reduced soils (Eh = -100 to -300 mV) (Ramesh and DeLaune, 2008). Soil redox potential is partly controlled by microbial activity and the supply of carbon and the primary electron acceptor, oxygen (Fiedler et al., 2007). Under limited oxygen partial pressures, as found in waterlogged soils, microbial activity switches from aerobic to selectively anaerobic respiration. Alternative electron acceptors are then utilized to enable respiration to continue, in the sequence: NO_3^-, MnO_2, $Fe(OH)_3$, SO_4^{2-} , and CO_2 or organic acids (Ramesh and DeLaune, 2008, Tack, 2010).

Metals and metalloids show contrasting behaviour under reducing conditions; they may become either more, or less, soluble as anaerobic conditions progress (Borch et al., 2010) due to a variety of reasons. For example, As tends to be more labile under anoxic condition, increasing As solubility and mobility. Reasons for this trend include the reduction of Fe^{3+} oxide to Fe^{2+} (Fig. 5.1) releasing sorbed arsenate, increasing pH values under anaerobic conditions which weakens arsenate adsorption strength on oxide surfaces, reduction of arsenate (As^{V}) to arsenite (As^{III}) which is much more soluble and minor effects such as increased formation of soluble Ca-arsenate complexes (Xu et al., 1991). By contrast, Cd can become less mobile under anoxic conditions because of the formation of CdS due to the reduction of SO_4^{2-} (Fig. 5.1) and increased adsorption at higher pH values (Tack, 2010); alternatively, large Fe^{2+} ion concentrations, from reduced oxides, can displace trace metal cations from humus adsorption sites into solution. Although biological activity has an important effect in modifying redox conditions that cause changes in element

valence and solubility it is seldom clear whether such changes are primarily chemical or biological (McBride, 2007).

Thus, changes in soil redox potential may have effects on Zn, Cd and Pb solubility and lability in soils which are difficult to predict. Reactions can be categorized into four main processes, including: (i) changes in soil pH, (ii) complexation and/or precipitation with sulfide and (bi-)carbonate, (iii) dissolution of Mn and Fe oxides and (iv) changes in adsorption on humus resulting from altered pH and greater competition with Fe^{2+} and Mn^{2+}.

(i) Under anaerobic condition, the soil pH alters toward neutral (pH 7), irrespective of initial pH, in both alkaline and acidic soils (Ponnamperuma, 1972, McBride, 1994). In acid soils, the increase in pH is related to consumption of H^+ ions by most reducing processes (e.g. Fe reduction; $Fe(OH)_3 + e^- + 3\boldsymbol{H^+} = Fe^{2+} + 3H_2O$) (see electrochemical reactions in Fig. 5.1). However, in alkaline soils, soil pH may be lowered by the accumulation of CO_2 gas and, consequently, produce carbonic acid (Fiedler et al., 2007). Therefore the metal solubility should be decreased in anaerobic acidic soils (Menzies, 2007).

(ii) Under anoxic soil conditions metal carbonate precipitation may occur due to increased CO_3^{2-} and HCO_3^- activity in the soil solution resulting from increased partial pressure of CO_2 and pH value (e.g. Zn;Ma and Lindsay, 1993). Under strongly reducing conditions and with even small activities of sulfide produced from reduction of SO_4^{2-} (Fig. 5.1), the metal is more likely to be precipitated as sulfide minerals (Tack, 2010). In such circumstances, if the ion activity product of metal carbonate or sulphides is exceeded, metal solubility, and lability, may be lowered (Menzies, 2007).

(iii) The oxides of Fe^{III} and Mn^{IV} provide one of the main adsorption surfaces for metals such as Zn, Cd and Ni under oxic condition, especially at pH values above 6.0. Reductive dissolution of those oxides may release those metals into the solution phase under flooding (Weber et al., 2009, Du Laing et al., 2009). In addition, the dissolution of Fe^{III} and Mn^{IV} oxides under reducing condition increases the concentration of Fe^{2+} and Mn^{2+} in the soil solution; both ions then compete strongly for remaining oxide adsorption sites, and sites on humus, also resulting in increased metal solubility. By contrast, (re-)oxidation will cause metal re-adsorption, on, and possibly co-precipitation in, newly formed, Fe/Mn oxides (Menzies, 2007). Although both Fe and Mn oxides must have an important influence on metal lability, Fe is the more interesting because of its greater abundance (Menzies, 2007).

(iv) The amount of organic matter present in the soil, and its degree of humification, can affect metal bioavailability by organic-complexation (Kashem and Singh, 2001) and also by promoting reduction through decomposition. Increased concentrations of dissolved organic ligands occur under reducing conditions because of release from (reduced) Fe oxides and production through (partial) anaerobic degradation processes (Ponnamperuma, 1972). Metal-organic complexes in the soil solution phase may be present as labile or non-labile colloidal particulates (e.g. Cu, see Ma et al., 2006) (see Chapter 3). Aerobic conditions (oxidation) promote the full mineralization of organic matter. This may release metals associated with organic fractions (Forstner, 1993), such as Cd, but is likely to lower solubility of metals that exist primarily in solution as fulvic acid complexes, such as Cu.

There is a need for greater understanding of the effects of reduction, and fluctuating redox status, on trace metal behaviour in soils. Isotopic dilution can

be used to follow changes in labile Zn, Cd, Pb and Fe under conditions of (i) progressive anaerobism and (ii) fluctuating redox conditions. An incubation experiment was established using samples of a contaminated grassland soil incubated under conditions intended to induce progressive and fluctuating redox regimes. The aims of the experiment and of this chapter were to:

(i) Examine the effect of redox conditions on mobilization of labile and non-labile Zn, Cd and Pb and determine the extent of transfer between these two pools;

(ii) Follow the dissolution of Fe oxide, and thereby its effect on metal lability and solubility, using enriched ^{57}Fe to determine labile adsorbed ferrous iron;

(iii) Assess the ability of WHAM(VI) to predict metal solubility under anaerobic conditions using isotopically exchangeable Zn, Cd, Pb and Fe as input variables.

5.2 Materials and methods

Most of the analytical methods used in this chapter are described in detail in Chapter 2. The following information describes the incubation experiment design and some additional information and methods not previously described.

5.2.1 Soil selection and experimental design

A soil sample was selected from a well-characterized grassland location at Clough Wood, Derbyshire, within the old Pb/Zn mining areas of Darley Dale (53^{o} 09’ 08” N, 1^{o} 36’ 57” W, Elev. 148 m; see section 3.2.1). The sampling location was in a small floodplain, adjacent to a stream in the base of the valley; the soil is therefore likely to be subjected to seasonal fluctuations in redox potential. A fresh soil sample (0 – 15 cm) was collected (see plate 3.1), transported to the laboratory in plastic bags, and stored at 5^{o}C for two days prior to setting up the

incubation experiments. A proportion of the soil sample was air dried, sieved and subjected to chemical analysis as described in section 2.1.2.

An investigation of short-term redox cycles consisted of 6 sequential reduction and oxidation cycles induced by submerging 5 g (dry mass equivalent) field-moist soil sample (moisture content originally 28%) in 30 mL 0.001 M $CaCl_2$ in 50 mL glass bottles with 24 replicates. The bottles were sealed using Zn-free rubber stoppers and incubated at 25^{o}C in a water bath with a gentle agitation. The first cycle (oxidation, T1; Fig. 5.2) was established by bubbling air through the suspension with a mechanical pump (Fig. 5.2) for 15 days. At the end of the first cycle (T1), 4 soil suspensions (R1-R4, Fig. 5.2) were removed and analysed. The remaining soil suspensions were then subjected to a reducing environment by replacing the air with CO_2-free N_2 gas for another 15 days. At the end of the second cycle (T2), again the next 4 soil samples were removed and analysed. This procedure was repeated six times for an overall 90 days.

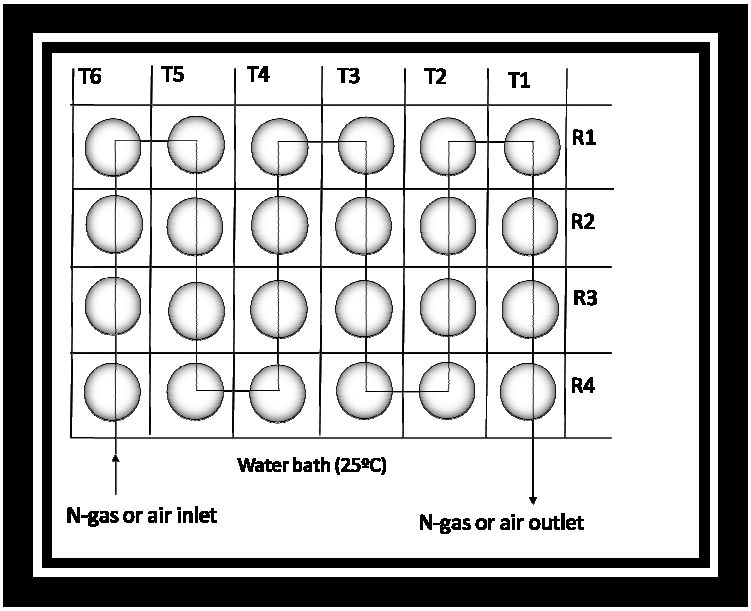

Fig. 5.2 Schematic presentation of the redox incubation experiment layout; T1-T6 represent sampling times for each cycle and R1–R4 represents the replicate bottles for each sampling time (T).

Longer term reduction and oxidation incubations were undertaken as described for each cycle but under either continuously reducing or oxidising conditions for the entire experimental period of 90 days with four soil replicates for each soil treatment. For the continuous reduction experiment, the soil suspensions were flushed with CO_2-free N_2 gas every day for 2 hours whereas in the corresponding oxidation experiment the soil suspensions were flushed continuously with air.

5.2.2 Isotope dilution technique

5.2.2.1 Recovery experiment

A preliminary recovery experiment was carried out to investigate whether ferrous iron, known to be equilibrated in a reactive form, can be correctly assayed by isotope dilution methods. Samples (0.5 g) of Na-saturated Amberlite IR-120 resin (Fisher Scientific Ltd, UK), enriched with 500, 280, 280 and 390 mg kg^{-1} of $Fe^{(II)}$, Zn, Cd and Pb respectively, were equilibrated in 100 mL conical flasks with 50 mL 10 mM $Ca(NO_3)_2$ for two hours. The resin suspensions were maintained under CO_2-free N_2 gas. An aliquot of 0.7 mL of enriched stable isotopes was then added from stock solutions (see section 2.1.1) with concentrations of 44.7, 16.9, 19.2 and 79.9 mg L^{-1} of $^{57}Fe^{(II)}$, ^{70}Zn, ^{108}Cd and ^{204}Pb, respectively and the resin suspensions were shaken for 2 hours under anoxic condition. The supernatant was separated as described in section 2.2.1 and isotopic ratios were measured immediately using ICP-MS (section 2.2.2).

5.2.2.2 Measuring E-values

The incubated soil suspensions to be assayed were spiked with enriched ^{57}Fe, ^{70}Zn, ^{108}Cd and ^{204}Pb solution two days before collecting the soil solution as shown in Table 5.1.

Table 5.1 The start, spiking and finishing dates for short-cycling and continuous redox experiments

Redox experiments	*Start date*	*Spike date*	*End date and analysis*
1st cycle (aerobic, T1)	15/07/2010	28/07/2010	30/07/2010
2nd cycle (anaerobic, T2)	30/07/2010	12/08/2010	14/08/2010
3rd cycle (aerobic, T3)	14/08/2010	27/08/2010	29/08/2010
4th cycle (anaerobic, T4)	29/08/2010	11/09/2010	13/09/2010
5th cycle (aerobic, T5)	13/09/2010	26/09/2010	28/09/2010
6th cycle (anaerobic, T6)	28/09/2010	11/10/2010	13/10/2010
Continuous aerobic (L_ox)	15/07/2010	11/10/2010	13/10/2010
Continuous anaerobic (L_Red)	15/07/2010	11/10/2010	13/10/2010

The spike solution added to all the soil suspensions was equivalent to approximately a 25 % change in the natural abundance of the labile pool of the isotopes ^{57}Fe, ^{70}Zn, ^{108}Cd and ^{204}Pb based on the E-values for Zn, Cd and Pb measured in the Clough Wood Grassland soil (CW-G; Chapter 3) and Fe oxide content measured in the reducing step of the sequential extraction procedure (SEP) (section 2.3). An aliquot (1 mL) of the working spike solution was added to each suspension with concentrations of 179 mg $^{57}Fe\ L^{-1}$, 27.7 mg $^{70}Zn\ L^{-1}$, 7.67 mg $^{108}Cd\ L^{-1}$ and 190 mg $^{204}Pb\ L^{-1}$. The contact time between the equilibrated soil suspension and the spike solution was two days both for the short-cycling and the continuous incubation experiments. When the spiked and equilibrated soils suspensions were processed, the Eh and pH values in the soil suspension were measured (section 5.2.3) and then a portion of the soil solution was removed in a 20 mL plastic syringe and filtered using 0.2µm Millex® Millipore filters. The filtrate was divided into three portions. The first (10 mL)

was acidified to a final concentration of 2% HNO_3 prior to assay of soluble metal and isotope ratios by ICP-MS (sections 2.1.2 and 2.2.2), the second (5 mL) was used directly to measure soluble anions and DOC (section 5.2.4) and the last aliquot (5 mL) was used to measure NLC metal using Chelex-100 (section 2.2.2). All measurements were undertaken as soon as possible after sampling. The isotopic ratios of Fe, Zn, Cd, and Pb were measured in three replicates of the control soil (unspiked) to enable calculation of E-values as described in sections 2.2.2 and 2.2.3. The sequence of isotope analysis and quadrupole dwell times used in the spiked and unspiked samples were as follows: ^{55}Mn (10 ms), ^{56}Fe (2.5 ms), ^{57}Fe (10 ms) ^{63}Cu (5.0 ms), ^{66}Zn (2.5 ms), ^{70}Zn (10 ms), ^{107}Ag (5.0 ms), ^{108}Cd (10 ms), ^{111}Cd (2.5 ms), ^{202}Hg (10 ms), ^{204}Pb (10 ms), ^{206}Pb (2.5 ms), ^{207}Pb (2.5 ms) and ^{208}Pb (2.5 ms).

5.2.3 Measuring soil suspension pH and redox potential (Eh)

Soil pH was measured in the soil suspensions at the end of short-cycle and continuous incubation experiments under air or N_2 flushing according to the redox conditions as described in section 2.1.2. The soil redox potential (Eh, mV) was determined using combined platinum (Pt) and reference (Ag/AgCl) electrodes with a Jenway 3010 voltmeter. The potential of the reference electrode (Eh_{ref}) was determined from measured Eh values (Eh_{meas}) in saturated suspensions of quinhydrone in pH buffers of 4.01 and 7.0. The measurement of soil Eh was undertaken by inserting the combined Pt electrode into the surface of soil slurries under either air or N_2 gas.

5.2.4 Anion analysis

Concentrations of major anions (F, Cl, NO_3, SO_4, and PO_4) in the filtrates were measured with ion chromatography. The analysis was undertaken using a Dionex DX500 with IonPac AS-14, 4 x 250 mm anion exchange column and with a mobile phase eluent mixture of 3.50 mM Na_2CO_3 and 1.0 mM $NaHCO_3$.

5.2.5 Prediction of metal solubility using WHAM(VI)

A description of the WHAM(VI) geochemical model used to fractionate and speciate the whole soil-solution system is presented in section 2.4. To predict metal solubility in the soil suspensions using WHAM(VI), six scenarios for input variables were tested, mainly to examine the ability of WHAM(VI) to deal with anaerobic systems involving substantial changes in $Fe^{(III)}$ and $Fe^{(II)}$ data. The input file was as described in section 2.4 but with the addition of anion concentrations measured in the solution phase (Cl, SO_4, F, and PO_4) and the E-values of Zn, Cd and Pb presented as 'total concentrations', (mol L^{-1} of suspension). The input of Fe to WHAM(VI) followed six alternative scenarios:

(i) Including Fe oxide determined as described in section 2.1.2. as a particulate phase (case 1)

(ii) Including the activity of Fe^{3+} in solution calculated from the pH based on the solubility product of $Fe(OH)_3$ (Tipping et al., 2003) + (i) (case 2).

(iii) Including measured Fe(II) presented as a total concentration ***in solution*** + (ii) (case 3).

(iv) Including an E-value for ferrous iron presented as a total concentration ***in the suspension*** + (ii); (case 4).

(v) Scenario (iv) but with Fe oxide as a variable input value for each data point based on the assumption that the labile pool of Fe(II) was dissolved from Fe oxide; therefore the Fe-oxide content in each sample was calculated by subtracting the Fe E-value from the original Fe oxide content (case 5).

(vi) Scenario (v) but with ferrous iron input as Fe(II) concentration in solution rather than total labile pool of Fe(II) (case 6).

5.3 Results and Discussion

5.3.1 Soil characterisation

The grassland soil (Grassland; GL) used in the redox trial was slightly acidic (pH = 6.12). Loss on ignition was 20.0% and the total concentrations of Fe, Zn, Cd and Pb were 34200, 5290, 66.2, 9660 mg kg^{-1} respectively. The initial soil Eh value, at the start of the incubation experiments (at zero time) was +500 mV. The iron oxide content, measured from the reducing step of the sequential extraction procedure (section 2.3) was 3990 mg kg^{-1} (11.7 % of the total soil Fe content).

5.3.2 Changes in pH, Eh and DOC

Figure 5.3 represents pH and redox potential development in soil suspensions subject to short term redox cycling trials and held under continuously aerobic or anaerobic conditions. Following the onset of incubation Eh never returned to the initial value of the original soil sample (+500 mV at zero time), but fluctuated between +400 and +172 mV and pH 5.3 and 6.6 as a result of changes in aeration (Fig. 5.3). There was a general increase in pH with decreasing redox potential (Fig. 5.4; $r = -0.86$; $P < 0.001$) with the suspensions subject to alternating aeration conditions falling between the continuously aerobic and anaerobic suspensions. This is in agreement with expected trends (Ponnamperuma, 1972, Hesterberg, 1998, Miao et al., 2006, Fiedler et al., 2007).

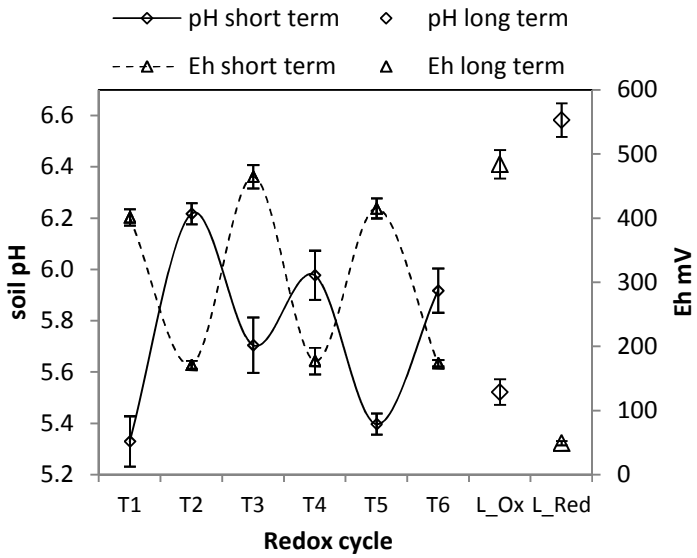

Fig. 5.3 Changes of soil pH and redox potential (Eh) as a function of alternating redox cycles; T1 to T6 represent short term incubation at 25°C where odd and even numbers show the oxidation and reduction cycles in sequence and L_Ox and L_Red represent suspensions held under continuous aerobic and anaerobic conditions, respectively. The error bars show the standard error of four replicates.

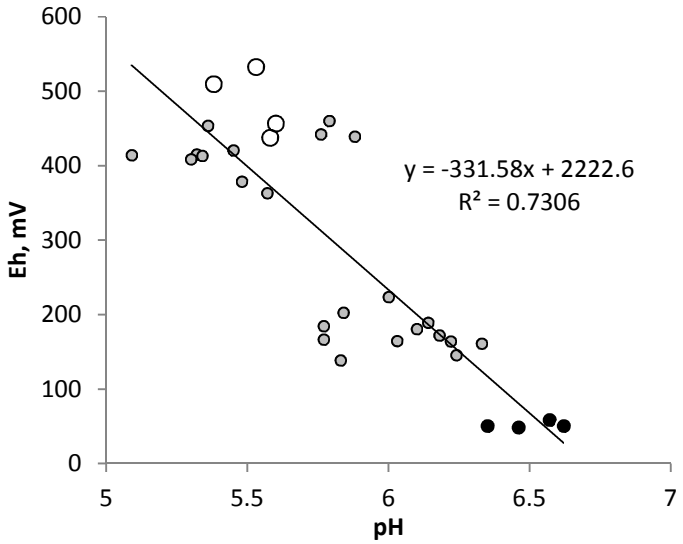

Fig. 5.4 Soil suspension pH as a function of Eh during incubation at 25°C. The black and white symbols represent the continuously reduced and oxidized suspensions, respectively, whereas the grey symbols represent suspensions subject to alternating redox status.

The soil used was from a site adjacent to the original mine shaft at Clough Wood (Plate 3.1) and contained 0.2% carbonate due to contamination with calcareous minespoil material. However, the site was under permanent grass and had relatively high organic matter content (20% LOI). Therefore the soil pH under aerobic conditions (c. pH 6.0) resulted from a combination of these characteristics (section 3.3.1). However, under reduced conditions, the pH of soils tends towards neutral values (Ponnamperuma, 1972) due mainly to the consumption of H^+ ions in reduction processes, balanced by a build up of CO_2 from organic matter decomposition (section 5.1).

The slope of the observed Eh/pH relationship depends partly on the proton-electron stoichiometry (n_H/n_e) of the redox reactions poising the soil system as shown from a well known Nernst equation (eq. 5.1).

$$Eh = Eo - \frac{RT\ln(10)}{F}\frac{n_H}{n_e}pH \approx Eo - 59\left(\frac{n_H}{n_e}\right)pH \quad (\text{at } 25^oC) \qquad (eq.5.1)$$

For example, for ferric hydroxide reduction to ferrous iron, the (n_H/n_e) stoichiometry is 3 and so the slope would be expected to be approximately 177 mV pH^{-1} (59 x 3) at 25°C. However, the presence of non-redox proton buffer reactions, such as H^+ adsorption on humus, ensures that the slope of pH against Eh is always greater than predicted from redox reactions alone (Menzies, 2007). For instance, it has been found that the soil solution Eh/pH slopes were -504 mV pH^{-1} during redox cycling (Miao et al., 2006), -359 mV pH^{-1} in oxidized fresh water sediment (DeLaune RD and Smith, 1985) and between -206 and -256 mV pH^{-1} in acid sulphate soil solutions (Charoenchamratcheep et al., 1987). In the current study, the Eh/pH slope was approximately -330 mV pH^{-1} (Fig. 5.4).

Figure 5.5 shows the changes in DOC (mg L^{-1}) and Eh (mV) during the redox incubation experiments. Generally, DOC values significantly increased ($P < 0.001$) with decreasing soil Eh (Fig. 5.6) and fluctuated with Eh value in the redox cycling trial. The highest DOC concentration (204 ± 11.6 mg L^{-1}) was

measured in the continuously reduced soil suspension with the lowest Eh value (50 ± 3.39 mV). This trend suggests reversible release and re-adsorption of humic or fulvic acids on Fe or Mn oxides with fluctuating redox conditions (Ponnamperuma, 1972). Partial decomposition reactions under anaerobic conditions followed by complete mineralisation to CO_2 during the aerobic cycle may also have contributed to the pattern shown. Sinha (1972) suggested that increase in DOC concentration in the soil solution results from anaerobic degradation processes.

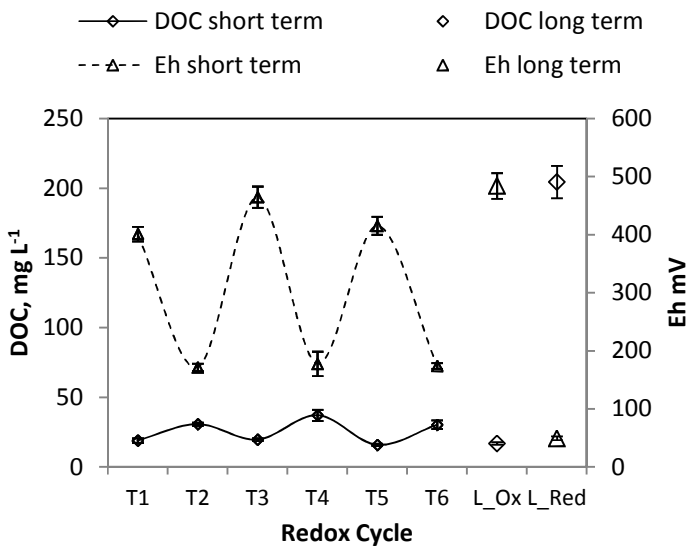

Fig. 5.5 Changes in dissolved organic carbon (DOC) and redox potential (Eh) as a function of redox cycles; T1 to T6 represent short term incubation at 25°C where odd and even numbers show the oxidation and reduction cycles in sequence and L_Ox and L_Red represent continuous incubation under aerobic and anaerobic conditions, respectively. The error bars show the standard error of four replicates.

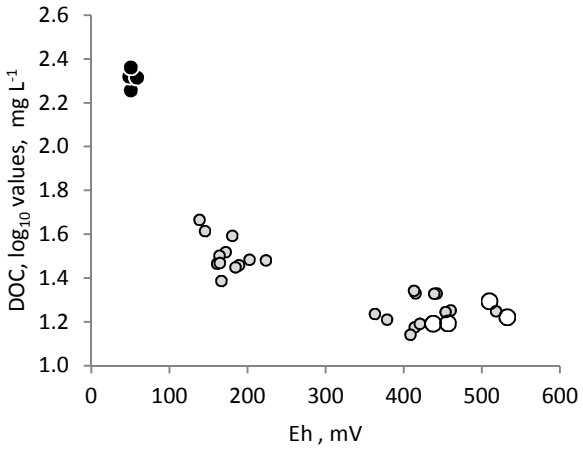

Fig. 5.6 Dissolved organic carbon (log_{10} values) in soil solution as a function of soil Eh during redox incubation at 25°C. The black and white symbols represent the continuously reduced and oxidized suspensions, respectively, whereas the grey symbols represent suspensions subject to alternating redox status.

5.3.3 Changes in solubility and lability of metals

Iron (Fe)

Seasonal variation, and shorter term fluctuations, in water table levels in soils causes Fe to transfer between oxidized and reduced forms (Fiedler et al., 2007). In the current study, two weeks was sufficient to produce marked alteration of redox potential with Eh values under reducing conditions ranging from +172 to +49.3 mV. Conversion of Fe^{3+} to Fe^{2+} requires Eh values below +180 to +150 mV, while the change from Mn^{4+} to Mn^{2+} occurs in the range +280 to +220 mV (Prasad, 2011). Moreover, Vepraskas (2001) suggested that, in many soils, a saturation period of around two weeks is likely to cause reduction of Fe.

The reduced Fe^{2+} and Mn^{2+} species exhibit a much greater solubility, as verified from the pattern of increasing and decreasing soluble Fe and Mn concentrations measured in the soil solution during reducing and oxidizing cycles, respectively (Fig. 5.7). Both Fe and Mn showed greater variation in solubility during redox cycles than other trace metals. Fig. 5.8 shows that a significant increase ($P < 0.001$) in soluble Fe was coincident with a decrease in Eh ($r = -0.79$) (Patrick et

al., 1996) and an increase of both pH (r = 0.80) and DOC (r = 0.94) (Table 5.2). Only at Eh values <-130 mV, would a loss of soluble iron be expected due to the formation of pyrite (FeS) (Miao et al., 2006) – well below the range of redox potentials achieved in the current experiment. As expected, DOC appeared to enhance the solubility of Fe (Fig. 5.8,), due to complex formation between fulvic acid and Fe^{2+} ions (Olivie-Lauquet et al., 2001).

Table 5.2 Correlation matrix for pH, Eh (mV), DOC (mg L^{-1}), metal E-values and soluble anion and cations (µg L^{-1}) measured during the incubation experiments (n = 32). E-values are presented as a proportion (%) of the total soil elemental concentration and, for Fe, also as a % of the reducible iron oxide-Fe concentration (%E Fe_{ox}). Bold Figures represent significant values.

	pH	*Eh*	*DOC*	*%E Pb*	*%E Cd*	*%E Zn*	*%E Fe_T*	*NO_3*	*SO_4*	*Mn*	*Fe*	*Zn*	*Cd*	*Pb*
Eh	**-0.86**													
DOC	**0.71**	**-0.66**												
%E Pb	-0.08	0.04	-0.02											
%E Cd	**-0.76**	**0.53**	**-0.64**	**0.48**										
%E Zn	**-0.67**	**0.49**	**-0.62**	**0.61**	**0.92**									
%E Fe_T	**0.81**	**-0.84**	**0.86**	0.12	**-0.61**	**-0.52**								
NO_3	**-0.74**	**0.76**	**-0.56**	**0.40**	**0.65**	**0.74**	**-0.63**							
SO_4	**-0.46**	**0.53**	**-0.31**	0.28	**0.43**	**0.51**	**-0.45**	**0.67**						
Mn	**0.81**	**-0.87**	**0.62**	0.22	**-0.49**	-0.34	**0.85**	**-0.49**	**-0.41**					
Fe	**0.80**	**-0.79**	**0.94**	0.04	**-0.68**	**-0.60**	**0.96**	**-0.64**	**-0.41**	**0.77**				
Zn	**-0.63**	**0.68**	**-0.50**	**0.41**	**0.58**	**0.68**	**-0.58**	**0.92**	**0.86**	**-0.44**	**-0.58**			
Cd	**-0.66**	**0.69**	**-0.55**	**0.33**	**0.62**	**0.67**	**-0.66**	**0.92**	**0.82**	**-0.47**	**-0.65**	**0.97**		
Pb	0.25	-0.17	**0.67**	0.30	-0.24	-0.11	**0.46**	0.19	0.25	**0.32**	**0.53**	0.25	0.18	
%E Fe_{Ox}	**0.80**	**-0.83**	**0.86**	0.12	**-0.61**	**-0.52**	**1.00**	**-0.62**	**-0.45**	**0.84**	**0.96**	**-0.58**	**-0.65**	**0.46**

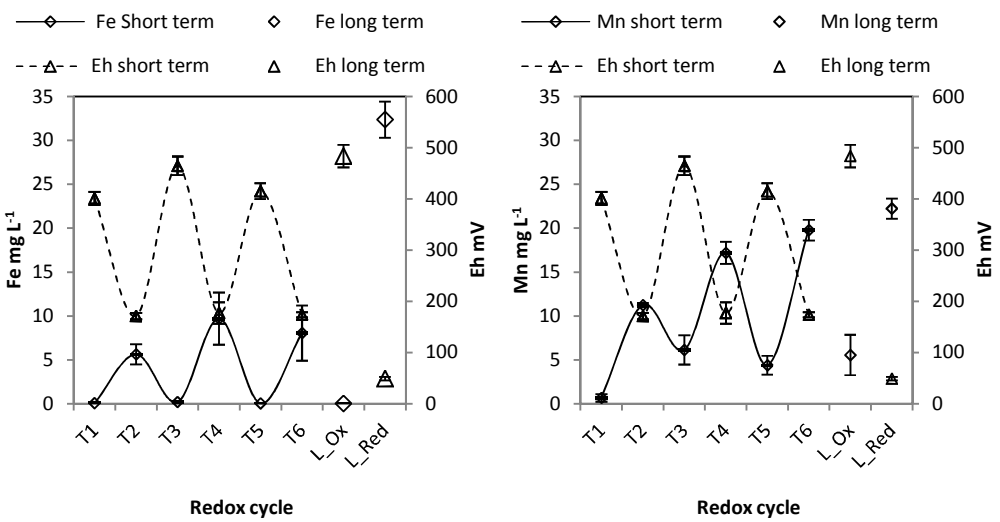

Fig. 5.7 Changes in soluble Fe and Mn concentration as a function of redox cycles; T1 to T6 represent short term incubation at 25°C where odd and even numbers show the oxidation and reduction cycles in sequence and L_Ox and L_Red represent continuous incubation under aerobic and anaerobic conditions, respectively. The error bars show the standard error of four replicates.

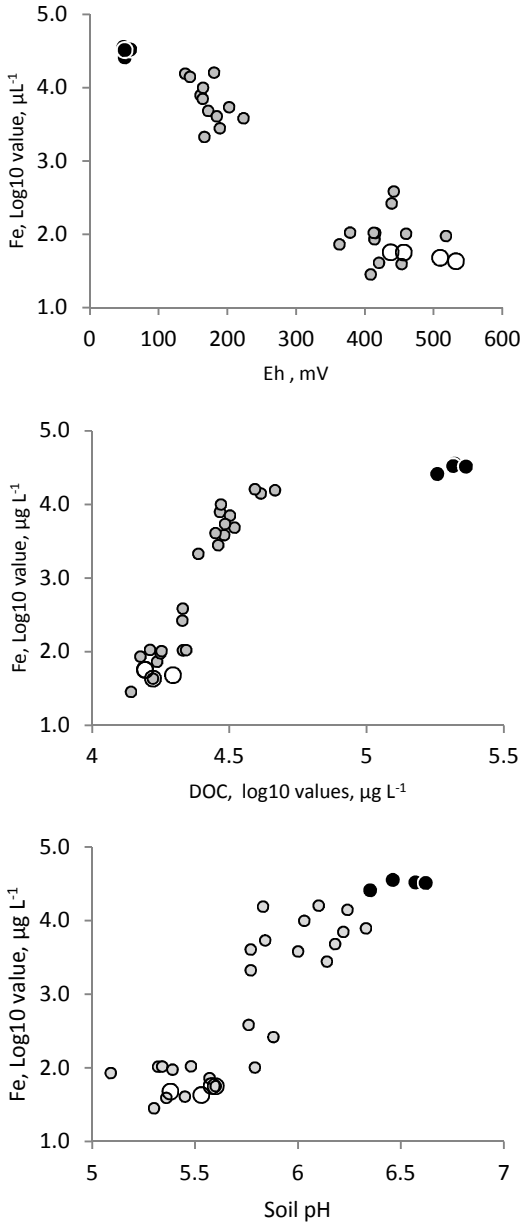

Fig. 5.8 Soluble Fe concentration as a function of Eh, DOC and pH during redox incubation at 25°C; the black and white symbols represent the continuously reduced and oxidized suspensions, respectively, whereas the grey symbols represent suspensions subject to alternating redox status.

The reactive forms of Fe oxide are the main reservoir of potentially reducible (and soluble) Fe but this phase is difficult to directly characterize under aerobic conditions because most iron in soils is present as Fe(III) in combination with oxygen. Under anaerobic conditions the reactive Fe will partially solubilize, and

this can be measured directly, but most of the Fe^{2+} ions produced will be strongly adsorbed by soil constituents. Stable isotope dilution with $^{57}Fe^{2+}$ may offer a means of quantifying reactive Fe under anaerobic conditions because it effectively assays both the Fe^{2+} that is in solution and that which is adsorbed but in dynamic equilibrium with the solution phase. To ensure that all the chemically reactive forms of Fe^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} were accessible to isotopic dilution, a recovery experiment was first undertaken under anoxic conditions using Amberlite IR 120 as described in section 5.2.2.1. Following the optimized approach described in sections 2.2.2 and 5.2.2.2, Fe, Zn, Cd and Pb in the resin suspensions showed average % lability values of 97.9, 98.3, 99.9 and 95.0 with standard errors of 0.36, 0.80, 0.29 and 3.15, respectively, confirming the validity of the ID approach to simultaneously probe the isotopically exchangeable pool of Fe, Zn, Cd and Pb.

To measure E-values in the soil suspensions, the Chelex-100 purification step (section 2.2.2) was used to measure chemically reactive Fe^{2+} because the presence of suspended ferric/ferrous particulate species presents a substantial risk. The existence of suspended nano-particles (< 200 nm diameter) would reduce the apparent isotopic abundance of the ^{57}Fe in solution and thereby result in E_{soln} values greater than E_{resin} values. Fig. 5.9 shows that E-values measured in both the resin and solution phases showed a 1 : 1 relationship under reducing conditions ($r = 0.999$; slope = 0.999). By contrast, the same measurements in both phases under oxidized conditions showed an overestimation of E_{soln} values ($r = 0.747$; slope = 0.397).

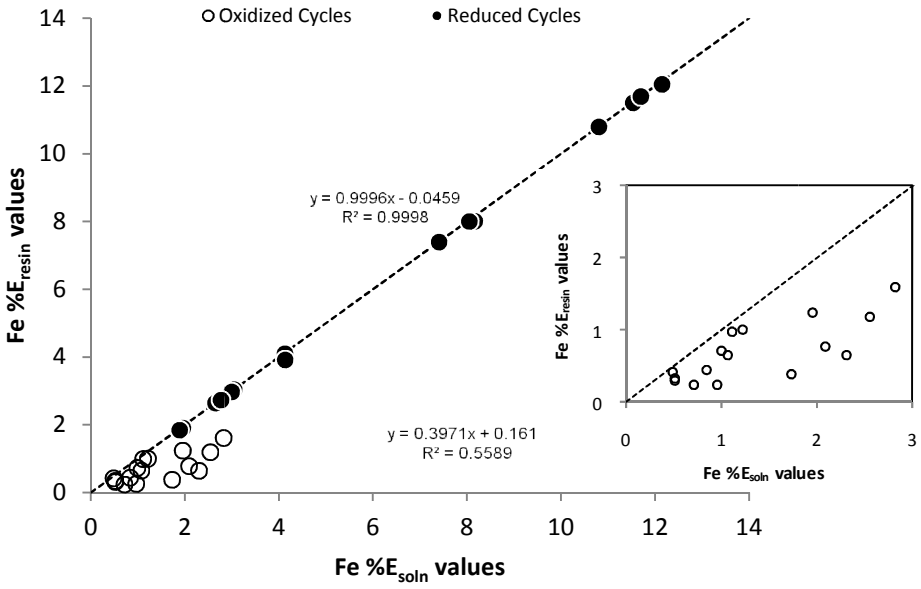

Fig. 5.9 Comparison of the isotopically exchangeable pool of Fe measured as E_{soln} and as E_{resin} to demonstrate the occurrence of non-labile colloidal metal; open (n = 16) and closed (n = 16) symbols represent oxidized and reduced phases of the redox cycles. Aerobic samples are also shown as a graphical inclusion. The broken line is the 1 : 1 relation.

The general % lability of Fe under oxidised conditions ranged from only 0.48 to 2.81% (Fig. 5.10) suggesting, perhaps, a very low level of isotopically exchangeable ferrous iron. However, it is difficult to interpret these results unequivocally because of the possibility of redox transformation of the $^{57}Fe^{2+}$ isotope. If, under aerobic conditions, the isotope achieved both chemical and redox equilibria then it is valid to equate the E-value to a mixed pool of labile ferrous and ferric iron. This assumption would be supported by the findings of Collins and Waite (2009) who measured the isotopically exchangeable pool of Fe in 21 soil samples. They found that a spiked ^{55}Fe radio-isotope was rapidly self-exchanged between Fe^{2+} and Fe^{3+}. The authors proved their findings by investigating the equality of the distribution coefficient (Kd) values of the spiked Fe isotopes added as either $^{55}Fe^{2+}$ or $^{55}Fe^{3+}$ in the soil-solution phases.

Figure 5.10 shows the changes in $\%E_{resin}(Fe_T)$, the E-value for Fe expressed as a % of the total soil Fe content (section 2.1.2), as a function of the redox cycles. The results showed that the % lability of Fe decreased and increased with increasing and decreasing Eh, respectively. Significant correlations ($P < 0.001$) were found between $\%E_{resin}(Fe_T)$ and soil pH ($r = 0.81$), Eh ($r = -0.84$) and DOC ($r = 0.86$) (Table 5.2). However, considering that only a 'reactive' iron oxide phase controls Fe redox geochemistry (Mitsunobu et al., 2008, Frierdich et al., 2011) the E-values were re-expressed as a proportion (%) of the apparent Fe oxide content measured in the reducing step of the SEP (section 2.3). Fig. 5.11 shows the E_{resin} values for Fe expressed as a proportion of both total Fe content ($\%E_{resin}(Fe_T)$) and (reducible) Fe oxide content ($\%E_{resin}(Fe_{ox})$). The results show that under continuously reducing conditions almost 100% of the Fe oxide content, determined from the SEP, had become isotopically exchangeable Fe^{2+}.

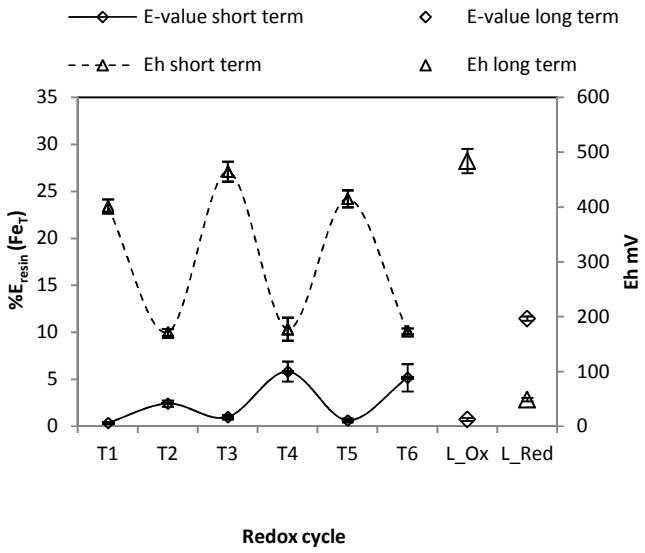

Fig. 5.10 Isotopically exchangeable Fe ($\%E_{resin}(Fe_T)$) as a function of redox cycling; T1 to T6 represent short term incubation at 25°C where odd and even numbers show the oxidation and reduction cycles in sequence and L_Ox and L_Red represent continuous incubation under aerobic and anaerobic conditions, respectively. Error bars show the standard error of four replicates.

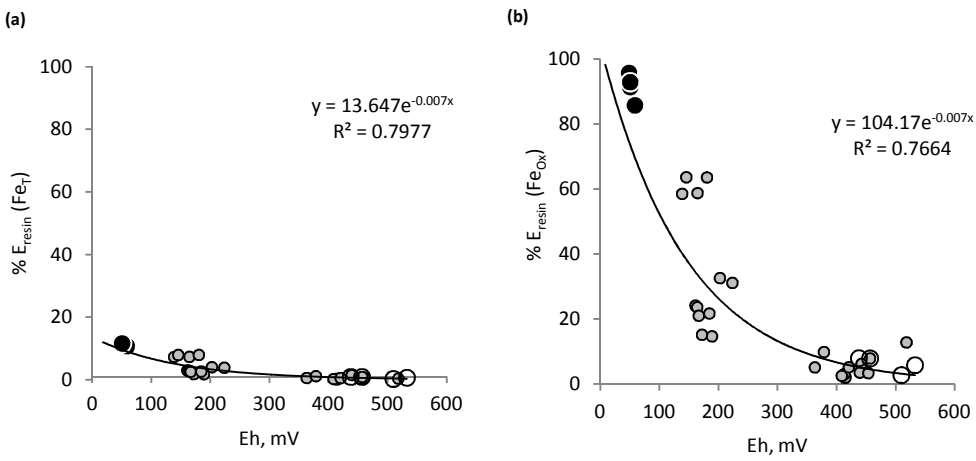

Fig. 5.11 Isotopically exchangeable Fe (a) as % of the total soil Fe content (%$E_{resin}(Fe_T)$ and (b) as % of the reducible Fe oxide content (%Eresin(Fe_{Ox}). The black and white symbols represent the continuously reduced and oxidized suspensions, respectively, whereas the grey symbols represent suspensions subject to alternating redox status.

The isotopically exchangeable pool includes reactive metal in both solution- and solid-phases (Collins and Waite, 2009). Fig. 5.12 shows the relationship between Fe concentration in solution and isotopically exchangeable Fe, both expressed as a proportion of the Fe oxide content: %$Fe_{soln}(Fe_{Ox})$ vs %$Fe_{resin}(Fe_{Ox})$. Traditionally, Fe^{2+} concentrations measured in the solution phase are used as a key indication of reductive Fe oxide dissolution. However, as hypothesised, the isotopic dilution results showed that most 'reactive' Fe^{2+} (c. 95%) was adsorbed on soil constituents rather than dissolved in the solution phase.

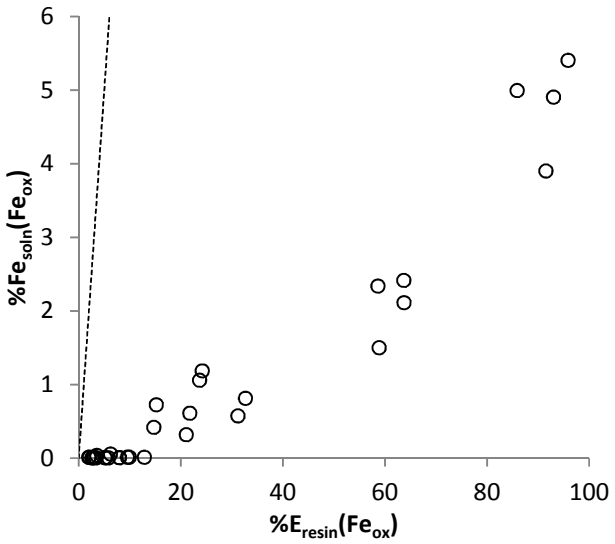

Fig. 5.12 Relationship between Fe concentration in solution and isotopically exchangeable Fe, measured during incubation and both expressed as a proportion of the Fe oxide content: $\%Fe_{soln}(Fe_{Ox})$ vs $\%Fe_{resin}(Fe_{Ox})$. The broken line represents the 1 : 1 relation.

The isotopically exchangeable pool of Fe can also be used to calculate the distribution of the labile Fe between solid-solution phases, (kd_L; L kg^{-1}), under reducing conditions. Fig. 5.13 shows the distribution coefficient (kd_L) for isotopically exchangeable Fe as a function of Eh and $\%E_{resin}(Fe_{ox})$. As expected, kd_L increased with decreasing Fe lability (Collins and Waite, 2009), and rising Eh value, as a smaller pool of labile Fe would be adsorbed on more strongly binding sites. This trend would be augmented by increasing DOC as redox potential fell (Fig. 5.6) by the formation of Fe(II)-organic complexes in solution but offset in part by the increase in pH under anaerobic conditions (Fig. 5.4) which would be expected to increase the strength of Fe^{2+} binding to solid phases.

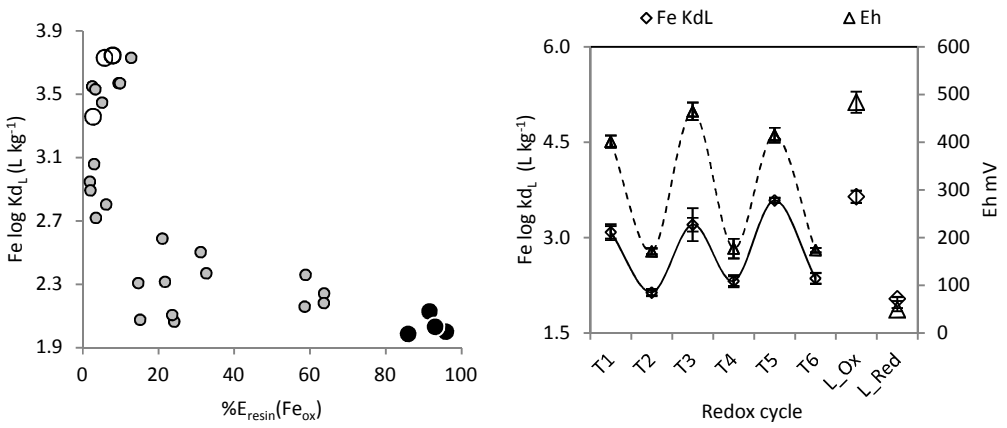

Fig. 5.13 Iron log kd_L (L kg^{-1}) as a function of (a) total labile Fe and (b) redox cycles (error bars show the standard error of four replicates). The black and white symbols in (a) represent the continuously reduced and oxidized suspensions, respectively, whereas the grey symbols represent suspensions subject to alternating redox status.

Zinc (Zn), Cadmium (Cd) and Lead (Pb)

Zinc and Cd concentrations in the solution phase of the suspensions generally increased and decreased during oxidising and reducing cycles, respectively, whereas soluble Pb concentration showed a continuous increase over the period of the incubation (Fig. 5.14). The overall increase in soluble metal concentrations at the end of the final short term redox cycle (T6) relative to the concentrations at the initial cycle (T1) were 60, 55 and 80% for Zn, Cd and Pb respectively (Fig. 5.14). Zinc and Cd concentrations increased under continuous oxidising conditions and decreased under anaerobic conditions, respectively. Lead concentration showed the reverse trend. In addition, Zn, Cd and Pb E-values generally increased and decreased during oxidising and reducing cycles respectively. However, the labile pool of Zn, Cd and Pb decreased as an initial effect of the decreasing Eh (T2) and then increased until the end of the short term redox cycling.

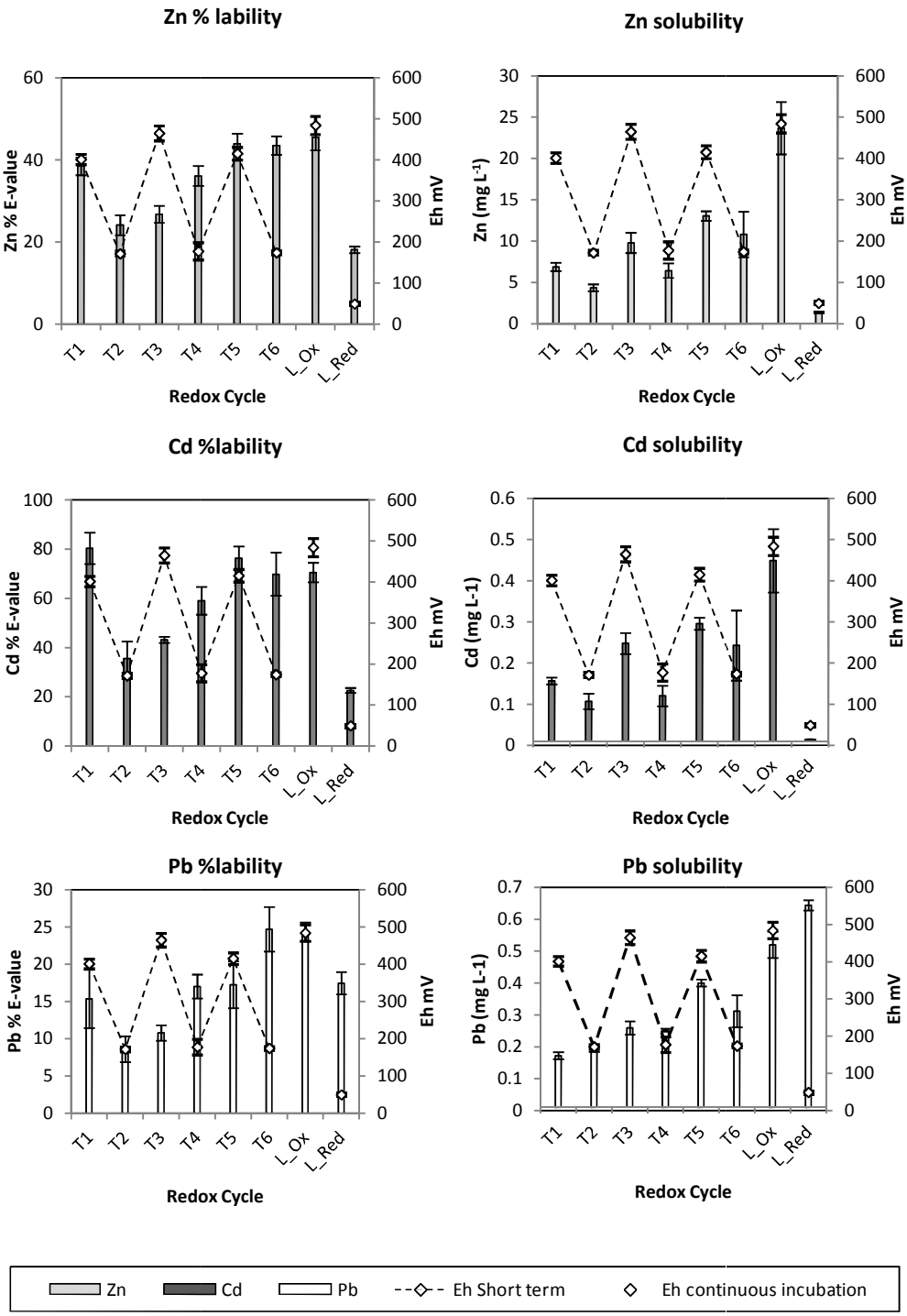

Fig. 5.14 Zinc, Cd and Pb solubility and %E values and redox potential as a function of redox cycling; T1 to T6 represent short term incubation at 25°C where odd and even numbers show the oxidation and reduction cycles in sequence and L_Ox and L_Red represent long term continuous oxidized and reduced incubated suspensions, respectively. Error bars show the standard error of four replicates.

Increasing solubility of Zn, Cd and Pb under oxidising conditions during (fluctuating) short term incubation, and of Zn and Cd under continuously oxidising conditions, may be explained mainly by the changes in soil pH. However, increasing Pb solubility is also likely to be affected significantly by higher DOC concentrations under continuous reducing conditions (Fig. 5.14) as it is well recognised that Pb is more strongly bound to soluble humus ligands (Sauve et al., 1997, Cances et al., 2003, Grybos et al., 2007). Miao et al. (2006) studied Zn solubility under both oxic and anoxic conditions by controlling soil pH (from 7.1 to 5.8) and Eh (from 500 to -200 mV). They found that Zn concentrations were mainly dependent on pH and were at a maximum under oxic conditions (at pH = 5.8). Fig. 5.15 shows that the lability of Zn and Cd also decreased with increasing soil pH, as found for Zn and Cd solubility (Table 5.2). This pattern of dependence of metal reactivity on soil pH is fairly well recognised in previous studies (Mench et al., 1994, Mench et al., 2000, Lombi et al., 2002, Cappuyns and Swennen, 2005).

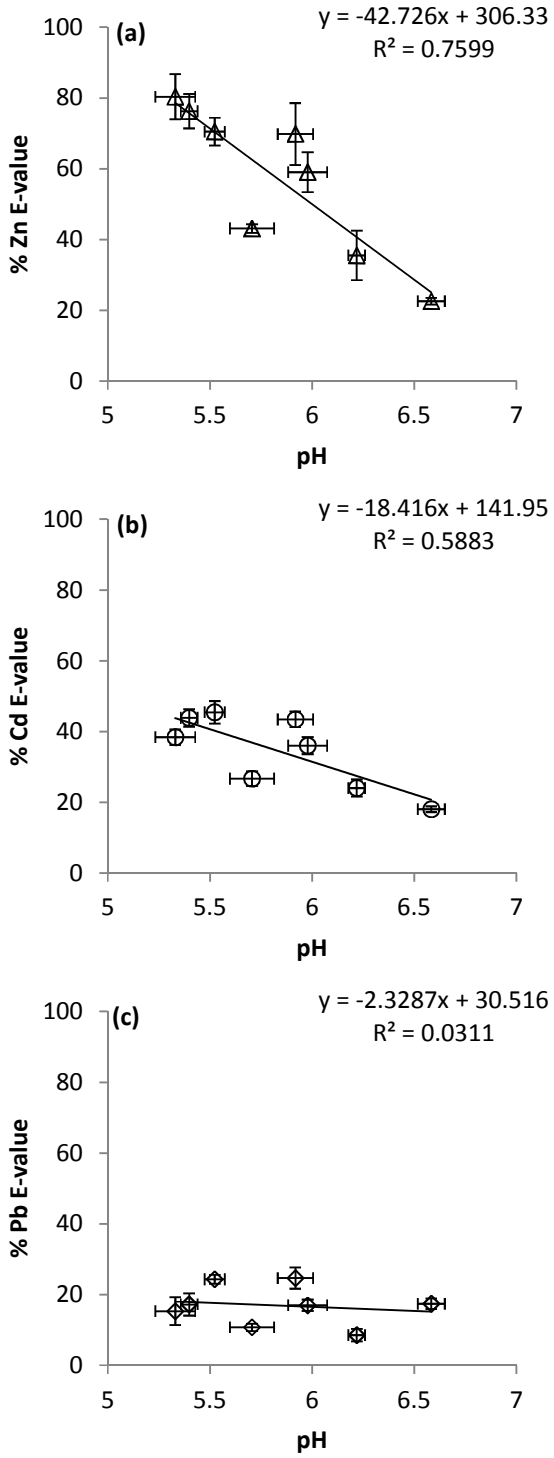

Fig. 5.15 Lability (%E value) of Zn (a), Cd (b) and Pb (c) as a function of pH during redox incubation at 25°C; error bars show the standard error of four replicates.

Can metal re-adsorption on Fe-oxide occur and lower metal lability?

Besides a simple relationship between pH-dependent adsorption strength and metal lability, other mechanisms may act to lower Cd, Zn and Pb lability under reducing conditions. For example, metals released from the reducible fraction of Fe may be re-adsorbed by the 'non-reducible' Fe fraction under reducing conditions (Mandal et al., 1988). This process might be active in the current study as the reducible Fe oxides represented only 11.7 % of the total soil Fe content. Cornu et al. (2009) stated that Pb dissolved under reducing conditions was probably re-adsorbed on the remaining Fe oxide phases. Moreover, an increase of Fe oxide specific surface area (and adsorption capacity) may occur due to reduction of Fe^{3+} on oxide surfaces (Contin et al., 2007).

Can metal precipitation occur causing lower metal lability?

Metal precipitation may occur as carbonates, phosphates and sulphides after release from Fe oxides (Ma and Lindsay, 1993, Tack, 2010). The saturation indices (SI) for carbonates derived from free ion activities predicted from WHAM(VI) (section 5.3.4) showed that metal-carbonates were under-saturated ($\log_{10}$(SI) < -0.5, data not shown). Formation of sulphides should not have occurred under even the lowest recorded Eh value of +50 mV which is higher than the Eh values required to form sulphides (Tack, 2010). The relatively low Pb lability found under reducing conditions may be caused by precipitation as chloropyromorphite ($Pb_5(PO_4)_3Cl$) following release of phosphate from reduced Fe oxides. The relative high PO_4 concentration measured in the soil suspension under continuously reducing conditions (250 µg L^{-1}) compared to that measured under continuously oxidizing conditions (below the detection limit, section 5.2.4) supports this possibility.

Can metal be occluded with re-precipitated Fe-oxides?

Fluctuations in redox potential might be expected to have additional effects on metal lability and solubility, notwithstanding the apparent reversibility shown by most of the measured variables across the sequence of redox cycles. It has been previously proposed that Zn, Cd and Pb could be incorporated into the crystal structure of Fe oxides (McBride, 1994, Contin et al., 2007, Menzies, 2007, Mitsunobu et al., 2008, Frierdich et al., 2011), as a consequence of repeated reduction-oxidation cycles (Palumbo et al., 2001, Contin et al., 2007) which might decrease the overall metal lability and toxicity. This hypothesis appears to be supported from data during the first three redox cycles (T1, T2 and T3; Fig 5.14). Comparing T1 (oxidized cycle) and T3 (re-oxidized cycle), the E-values of Zn, Cd and Pb decreased concurrently with re-oxidation of Fe oxides. The loss of metal lability after the three cycles suggests metal fixation may have occurred according to the occlusion hypothesis. Contin et al. (2007) also found an increase in metal retention on re-oxidized oxides following successive redox cycles. However, further repeated cycling (T4, T5 and T6; Fig 5.14) between Fe reduction and oxidation then appears to contradict the occlusion hypothesis as evidence for increased metal fixation disappeared. Although a decrease in metal lability was obtained after the third cycle (T3; 45 days) the subsequent redox cycles restored the original metal lability of Zn and Cd and even increased Pb lability by 60% at the end of the redox cycles (T6; Fig 5.14). The observed reversibility of chemically reactive Fe (Fig. 5.10) would also suggest that a process of progressive metal occlusion is unlikely to exist. Moreover, the basic concept that reduction leads to release of metals is contradicted by the fact that reduction causes an increase in pH resulting in lower metal lability as discussed in section 5.3.3. It has also been suggested that reductive Fe oxide dissolution may increase metal *bioavailability* (Weber et al., 2009, Du Laing et al., 2009). However, contrary to this observation, broad negative correlations with lability were observed for Zn ($r = -0.52$; $P < 0.001$) and Cd ($r = -0.61$; $P < 0.001$) with

progressive reduction of Fe oxides in the current study (Fig. 5.16) while no obvious trend was observed with Pb lability ($r = 0.12$; $P > 0.05$).

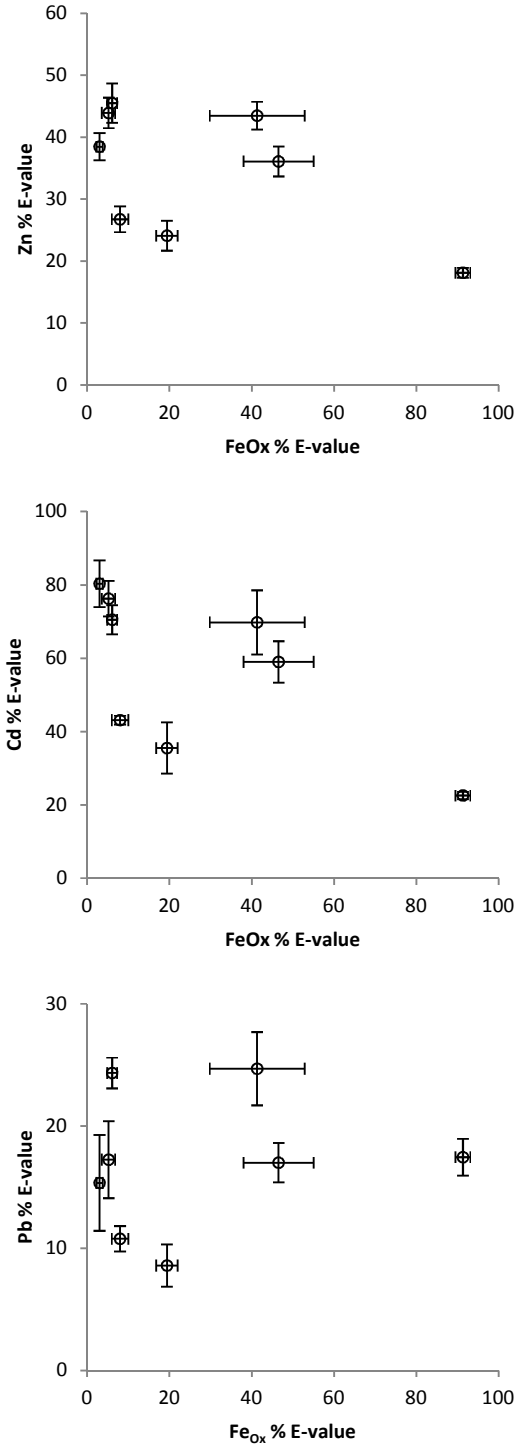

Fig. 5.16 Lability (%) of Zn, Cd and Pb as a function of Fe_{Ox} % E-value during redox incubation at 25°C; error bars show the standard error of four replicates.

5.3.4 Using Zn, Cd, Pb and Fe E-values with WHAM(VI) to predict metal solubility

Modeling chemical speciation is vital to predict metal solubility in soils. However, geochemical models such as WHAM(VI) show that metal species may be affected by the way in which Fe dynamics are described within the model. Iron must be considered both as Fe^{2+} and Fe^{3+} ions competing for adsorption sites and as a solid phase oxide providing adsorption sites for trace metal binding to soil. In this section, the dual role of Fe as a competitor ion and as an adsorption phase for Zn, Cd and Pb was tested using WHAM(VI). Incorporating isotopically exchangeable Fe as an input variable to WHAM(VI) to model trace metal solubility under fluctuating redox conditions has not been reported in the literature. Labile Fe can compete significantly with other metals to associate with humus (Guthrie et al., 2005) while Fe oxides are important due to their abundance in the soil (Menzies, 2007) and their high affinity for trace metals such as Zn, Cd and Pb (Buekers et al., 2008b), especially at high pH values. Both these factors are particularly important under fluctuating redox conditions during which there may be substantial changes in chemically reactive Fe^{II} and Fe^{III} and reductive dissolution or (re-) precipitation of Fe oxides (see section 5.3.3). To fully investigate the dual role of Fe on metal solubility, WHAM(VI) was run with six different cases as illustrated in Table 5.3 and described in section 5.2.5.

Table 5.3 Input of Fe to WHAM(VI) followed the six alternative cases described in section 5.2.5

CASE	(Fe^{3+}) activity from Ks (Fe_{ox})	Constant Fe-oxide content	Variable Fe-oxide content	Solution Fe^{II} concentration	Labile Fe^{II} (E-value)
1		√			
2	√	√			
3	√	√		√	
4	√	√			√
5	√		√		√
6	√		√	√	

Comparison of solubility models

Figure 5.17 represents measured and predicted values of Zn, Cd and Pb solubility using WHAM(VI) parameterized with the six proposed scenarios described in section 5.2.5 and Table 5.3. Comparisons of solubility models were based on RMSD (eq. 3.1), R-squared (R^2) and the mean deviation between measured and predicted values (ΔpM_{soln} as $\log_{10}$ values). Although, R^2 represents a correlation between measured and predicted values for each case it does not indicate whether absolute values agree well but provides a measure of how consistently the measured and modeled values co-vary.

In general, the prediction model in all studied cases showed a greater spread from the 1:1 line at lower metal concentration and high soil pH (Fig 5.17). Table 5.4 summarizes the values of R^2, RMSD and ΔpM_{soln} for the simulation of soluble Zn, Cd and Pb concentrations by the six proposed models described in Table 5.3. The RMSD and ΔpM_{soln} are both measures of the difference between modeled and measured values. Results for Zn, Cd and Pb showed that predicted values were generally within one log unit of measured data (Fig. 5.17). Generally, the RMSD ranged from 0.30 to 0.50 for all metals, indicating that WHAM(VI) adequately modelled metal solubility in the soil solution (Ge et al., 2005). However, the model based on Case 5 was more successful than the others for Zn according to the values of RMSD and ΔpM_{soln} (0.30 and 0.18 respectively). For Cd and Pb, the best results were achieved with Case 2; RMSD and ΔpMsoln values were 0.31 and 0.15 for Cd and 0.46 and 0.34 for Pb respectively.

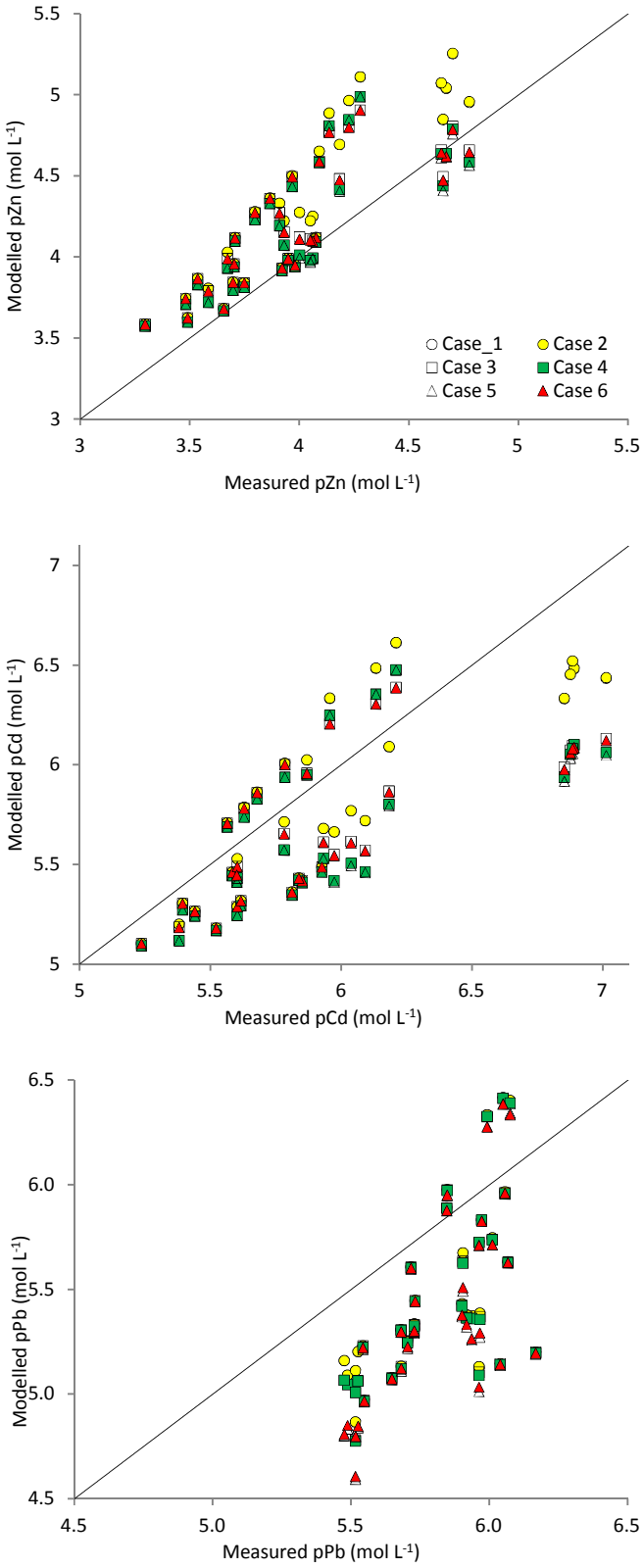

Fig. 5.17. Predicted versus observed soluble metal concentrations (mol L^{-1}, -$\log_{10}$ scale) in a 0.001 M $CaCl_2$ soil suspensions (n=32). Predictions were made with WHAM(VI), parameterized by 6 alternative cases (see Table 5.3). The solid lines represent the 1:1 lines.

Table 5.4. Values of R^2, RMSD, and *mean* deviation on a log scale (ΔpM_{soln}) for the simulation of soluble Zn, Cd and Pb concentrations (in a 0.001 M $CaCl_2$ extract) in soil suspensions (n = 32 for each metal). Predictions were made using WHAM(VI) with six proposed models described in Table 5.3.

metals	*WHAM(VI) Case*					
	1	2	3	4	5	6
			R^2			
Zn	**0.809**	0.810	0.693	0.653	0.637	0.656
Cd	0.690	**0.693**	0.524	0.481	0.465	0.495
Pb	0.388	0.389	0.420	0.417	**0.465**	0.443
			RMSD			
Zn	0.387	0.388	0.309	0.301	**0.301**	0.308
Cd	0.313	**0.312**	0.415	0.450	0.457	0.397
Pb	0.465	**0.464**	0.482	0.486	0.544	0.524
			ΔpM_{soln} (pM_{soln} predicted – pM_{soln} measured)			
Zn	0.312	0.313	0.224	0.188	**0.183**	0.219
Cd	-0.153	**-0.152**	-0.241	-0.275	-0.280	-0.245
Pb	-0.339	**-0.337**	-0.354	-0.358	-0.411	-0.405

Generally speaking, under aerobic condition, the concentration of Fe^{2+} will be very small and the Fe-oxides will be stable while under reducing condition the concentration of Fe^{2+} will increase due to reductive dissolution of Fe-oxide. Therefore, the effect of Fe^{2+} competition for binding sites is more likely to be greatest under anaerobic condition resulting in increasing metal solubility in the soil solution phase. In addition, the reductive dissolution of Fe-oxide will also contribute to increased metal solubility due to release the adsorbed metal and

loss of adsorption sites. The modelled results for Cases 1-6 were in general agreement with these assumptions. Table 5.5 shows a comparison of the six modelled values of Zn, Cd and Pb under both aerobic and anaerobic conditions to evaluate the effect of Fe^{2+} ion competition and loss of adsorption sites on Fe-oxide, respectively, on the model outcome. The comparison is based on the mean deviation (ΔpMsoln, $\log_{10}$ scale) and the mean % ratio (normal scale) of the six modelled cases relative to Case 1 which can be regarded as a control scenario in which Fe is not considered.

Table 5.5 The mean deviation on a $\log_{10}$ scale, ΔpM_{soln} (Case 1 – Case n) and the mean % ratio in parentheses (Case n/Case 1) for the six alternative models of Zn, Cd and Pb solubility in soil suspensions under both aerobic and anaerobic conditions. Case n represents the individual case numbers as shown in Table 5.3.

Case	*Zn*		*Cd*		*Pb*	
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
1	0.000 (100)	0.000 (100)	0.000 (100)	0.000 (100)	0.000 (100)	0.000 (100)
2	-0.001 (99.8)	-0.0021 (99.7)	-0.00085 (99.8)	-0.0016 (99.7)	-0.0014 (99.8)	-0.00071 (99.8)
3	0.0013 (100)	0.175 (128)	0.0013 (100)	0.175 (127)	0.00015 (100)	0.0298 (112)
4	0.0286 (106)	0.219 (151)	0.0273 (106)	0.218 (150)	0.0041 (101)	0.0339 (113)
5	0.0290 (106)	0.229 (154)	0.0277 (106)	0.226 (152)	0.0120 (103)	0.132 (158)
6	0.0018 (100)	0.182 (130)	0.0018 (100)	0.181 (128)	0.0079 (102)	0.124 (155)

The results show that under aerobic condition the inclusion of Fe^{2+} concentration, measured in the solution and/or as isotopically exchangeable and allowing for the loss of Fe-oxide content, appears to be irrelevant in all cases because of negligible effects on metal solubility (Table 5.5). Average modelled values for

metal concentration in solution, relative to Case 1, were close to unity for all three metals, ranging from 0.99 – 1.06. By contrast, the inclusion of Fe (either as solution concentration, isotopically labile Fe^{II} or variable Fe-oxide content) obviously showed significant effects on modelled values compared to case 1 under reducing condition, particularly in Case 5 and for all metals where isotopically labile Fe^{2+} and variable amounts of Fe-oxides were considered (Table 5.3). Therefore, the following discussion will focus on the results gained from anaerobic conditions. In the case of Zn and Cd, including Fe as an input variable to WHAM(VI) showed an average increase in modelled solution metal concentration in the following order: Case 5 > Case 4 > Case 6 > Case 3 > Case 2. These effects improved the modelled Zn values by pushing them towards the 1:1 line as shown in Fig. 5.17. By contrast, Cd results showed more scatter around 1:1 line than that of Zn and so results were more variable. The highest concentration of Zn and Cd was seen for Case 5 due to inclusion of both labile Fe^{2+} concentration and allowance for the loss of Fe-oxide under anaerobic conditions. The binding of labile Fe^{2+} by humus is expected to have a strong effect on binding of Zn and Cd.

Generally, all modelled values of Pb concentration in solution (i) were greater than measured values, (ii) were more displaced from the 1:1 line and (iii) showed greater scatter than the Zn and Cd data. Therefore, introducing competition from Fe^{2+} for adsorption sites, and allowing loss of Fe oxide binding sites, inevitably aggravated this error by increasing Pb solubility and causing greater deviation from the measured values. The average increase in modelled soluble Pb concentration followed the order: Case 5 > Case 6 > Case 4 > Case 3 > Case 2.

Unexpectedly, there was a substantial difference between Case 5 and Case 6 for the modelled Zn and Cd concentrations in solution: Case 5 predicted greater Cd and Zn solubility. This suggests that for Case 6, where Fe^{2+} measured in the

solution was used as an input variable, the (modelled) adsorbed labile Fe^{2+} was underestimated by WHAM(VI) (or vice versa for Case 5). Indirect evidence for this can be seen by comparing the modelled and measured values of Fe^{2+} in Case 5 (Fig. 5.18). Although the model predictions of (total) Fe^{2+} concentration in solution were quite good under reducing conditions (grey symbols, Fig. 5.18), it is clear that the predicted values of Fe^{2+} were generally greater than measured values in Case 5 (using isotopically exchangeable Fe^{II} as an input variable). In contrast, the difference between both Cases (5 and 6) was very small for modelled Pb values (Table 5.5), Taken together, this may suggest that competition from Fe^{2+} for adsorption sites was less important than loss of Fe oxide sites for Pb whereas the reverse was true for Cd and Zn. Cadmium and Zn are not likely to be substantially adsorbed on Fe oxide but may be subject to substantial competition from ferrous iron ions for adsorption sites on humus (see following sections).

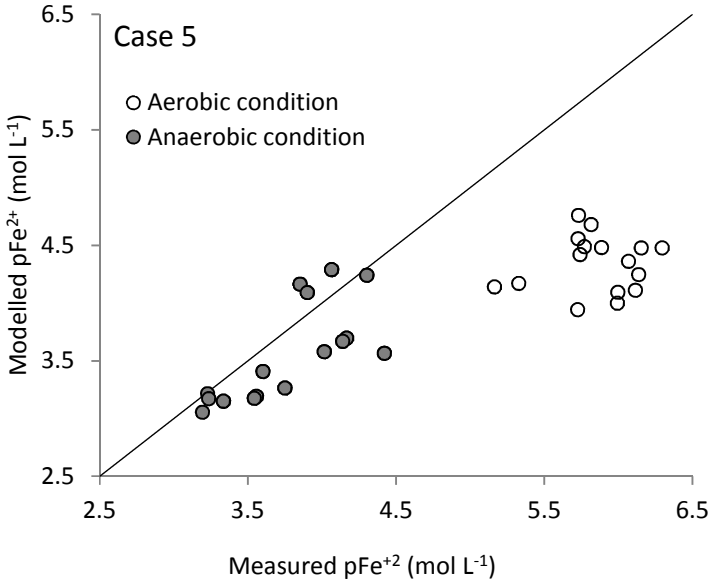

Fig. 5.18 Modelled versus measured soluble Fe^{2+} concentrations (mol L^{-1}, $-\log_{10}$ scale) in a 0.001 M $CaCl_2$ soil suspensions (n=32). Predictions were made with WHAM(VI), parameterized by Case 5, using isotopically exchangeable Fe^{II} as an input variable (see Table 5.3). The solid line represents the 1:1 relation.

The effect of loss of Fe-oxide content due to reductive dissolution can be observed by comparing Cases 5 and 4. The results showed that Zn and Cd were not affected by adsorption on Fe oxide surfaces where the ratio of the modelled values in Cases 5 and 4 relative to the modelled values in Case 1 (Table 5.5) were similar (~ 1.5). This is in line with the expected fractionations of Zn and Cd which are expected to associate mainly with humus (see next section). By contrast, Pb was significantly affected by Fe-oxide loss; the results in Table 5.5 show that the modelled Pb concentration ratios for Cases 4 and 5 (relative to Case 1) were 1.13 and 1.58, respectively. In Case 4 where Fe-oxide content was input to WHAM(VI) as a constant value, the adsorbed Pb was greater which reduced modelled Pb solubility (green symbols in Fig 5.17).

There appear to be errors in modelling Pb dynamics in WHAM(VI) which currently negate the expected advantages in refining the input variables for Fe (Fig. 5.17). It is likely that binding of Pb to iron oxide is underestimated or there may be other adsorption sites present which are not sufficiently accommodated (such as loss of Mn-oxides due to reductive dissolution). It was suggested by Bueckers et al. (2008b) that the underestimation of total surface area of Fe-oxyhydroxides in WHAM(VI) may lead to an underestimation of metal binding. Alternatively, there may be problems with the measurements of Pb concentration in solution, perhaps associated with rapid precipitation of Fe oxide, and adsorption of Pb, during the process of extracting, filtering and stabilising (acid) an anaerobic solution sample. It is certainly apparent that several researchers have reported poor agreement between measured and modelled Pb using WHAM(VI) (Weng et al., 2002, Schroder et al., 2005, Unsworth et al., 2006).

Metal fractionation as predicted by WHAM(VI)

Figure 5.19 shows the metal distribution on humic acid and Fe and Mn oxides as predicted by WHAM parameterized by Case 5 as a function of metal % lability for Zn, Cd and Pb. Case 5 was used to illustrate the metal fractionations because both labile Fe^{II} and loss of Fe-oxides were considered. It is clear that almost all labile Zn and Cd existed bound to humic acid across the range of conditions studied (> 90%). There was a small increase in the proportion of labile Zn and Cd in solution as the metal lability increased – probably associated with lower pH values. Since the majority of Zn (> 95%) and Cd (> 90%) were adsorbed to humic acid, it seems that both metals are affected by Fe^{II} competition for sites on humus rather than changes in the adsorption capacity of the Fe/Mn-oxides. By contrast, Pb varied substantially between the different fractions. The (much smaller) proportion of labile Pb was distributed more evenly between humus and oxide bound fractions; as Pb lability (Pb $\%E_{resin}$) increased a greater proportion was held by humic acid. A substantial Pb fraction was associated with Mn-oxides as seen in Fig 5.19. It has certainly been reported previously that Pb is strongly complexed by Mn oxides (Lofts and Tipping, 1998, Trivedi and Axe, 2000, Cances et al., 2003). However, this may be erroneous due to an overestimation of adsorption sites on Mn-oxides due to the assumed preservation of MnO_2 adsorption capacity under anaerobic conditions. In the current study, the reductive dissolution of Fe-oxides reached approximately 100% (i.e. complete dissolution of reactive Fe-oxide), particularly under continuous reducing conditions. Moreover, it is well known that Mn-oxides are more 'easily reduced' than Fe-oxides (Laing, 2010) in accord with the expected sequence of electrochemical reactions caused by progressive anaerobism (Fig. 5.1). Therefore, loss of Mn-oxides should be considered in predicting metal fractionation under reducing systems.

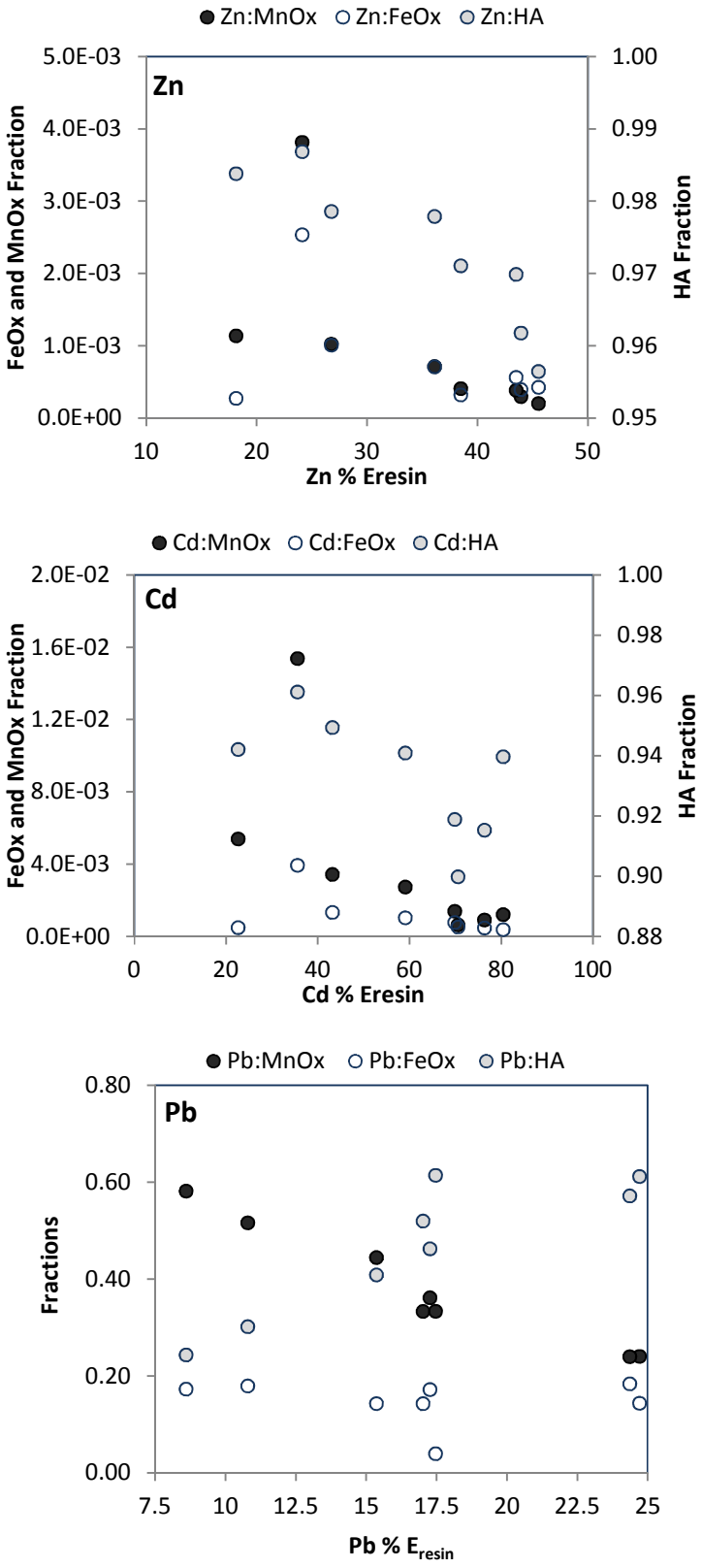

Fig. 5.19 Metal distribution on Humic Acid (HA) and Fe and Mn oxides (FeOx and MnOx) predicted by WHAM parameterized by Case 5, as a function of % metal lability (E_{resin}) for Zn, Cd and Pb. Note the two Y-axis scales for Zn and Cd.

Unlike Fe, Mn has no available stable isotope to measure its lability using ICP-MS. Therefore, the extent of reductive dissolution of Mn-oxide could not be measured for input to WHAM. However, to calculate the loss of Mn-oxides during the redox cycles, Mn concentration measured in the soil solution phase can be used to predict the labile concentration of Mn (the measured Mn in the solution + particle-bound concentration) as an output from WHAM(VI). The predicted labile Mn can then be used to calculate (by difference) the remaining Mn-oxide content, just as the remaining Fe-oxide was estimated from the measured labile Fe. The dataset incorporating loss of Mn-oxides predicted by WHAM(VI) was used to rerun Case 5 again (hereafter called Case 5(Mn)). Fig. 5.20 shows the resulting Pb fractionation associated with HA, Fe- and Mn-oxides after considering the loss of Mn-oxides predicted by WHAM(VI). The results show only a slight change in Pb fractionation for Case 5(Mn) compared to the model without variable Mn-oxide content. These changes also create a slight change in the Pb solubility as seen in Fig 5.21.

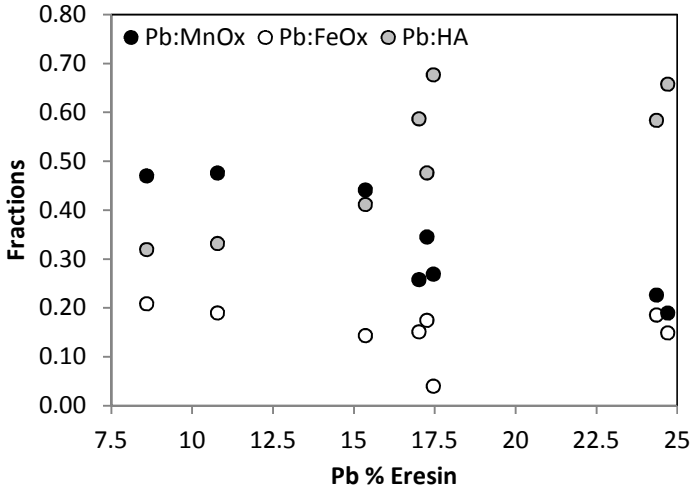

Fig. 5.20 Metal distribution on Humic Acid (HA), Fe and Mn oxides (FeOx and MnOx) as predicted by WHAM parameterized by Case 5(Mn) as a function of Pb % lability. Case 5(Mn) considers the loss of Mn-oxides as predicted by WHAM(VI) (see text for more details).

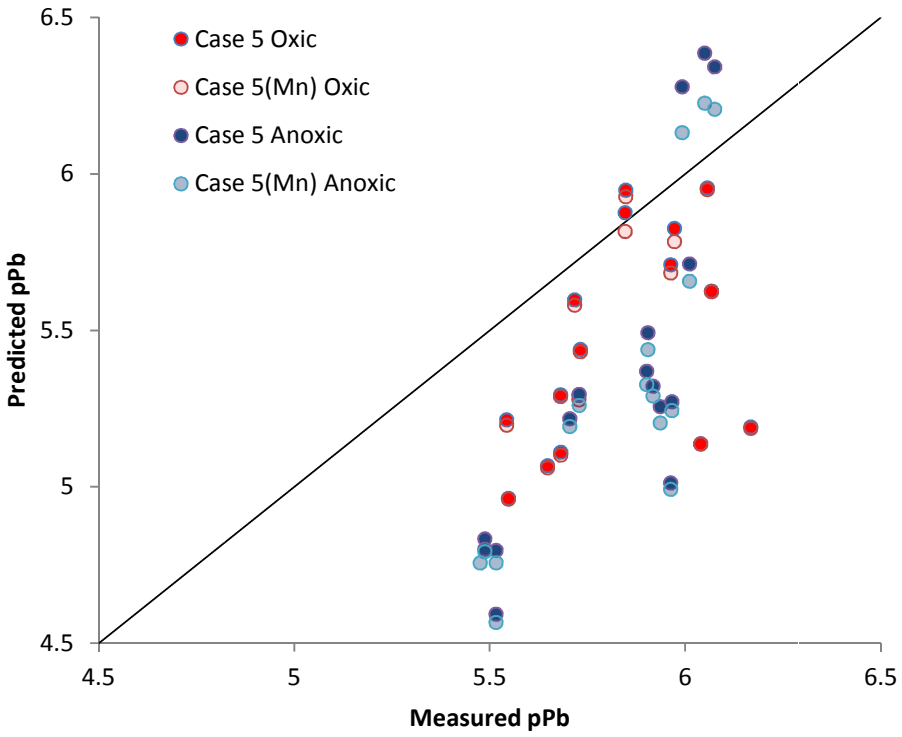

Fig. 5.21. Predicted versus observed soluble metal concentrations (mol L^{-1}, $-\log_{10}$ scale) in a 0.001 M $CaCl_2$ soil suspensions (n=32) under both aerobic and anaerobic conditions. Predictions were made with WHAM(VI), parameterized by Case 5 and Case 5(Mn) (see text for more details). The solid line represents the 1:1 relation.

The Pb solubility was slightly increased as a result of considering reductive dissolution of Mn-oxide. Unfortunately, for most data, this increased the error in model prediction of p(Pb). Furthermore, the relatively small change in Pb solubility (Fig. 5.21) and the continued importance of MnO_2 as a binding site under anaerobic conditions (Fig. 5.20) may point to an underestimation of labile Mn^{II} from measured concentration of Mn in solution. The maximum loss of Mn-oxides predicted by WHAM(VI) was ~25% compared to ~97% loss measured directly for Fe-oxides by isotopic exchange. It is therefore likely that WHAM(VI) underestimated the labile Mn pool from measured Mn concentration in the solution phase. As found for Fe (Fig. 5.12), Mn concentration in the solution

phase is likely to be very small compared to the adsorbed labile pool. Fig. 5.22 shows that the remaining Fe-oxide content, predicted from measured Fe in the solution phase, was overestimated by WHAM(VI).

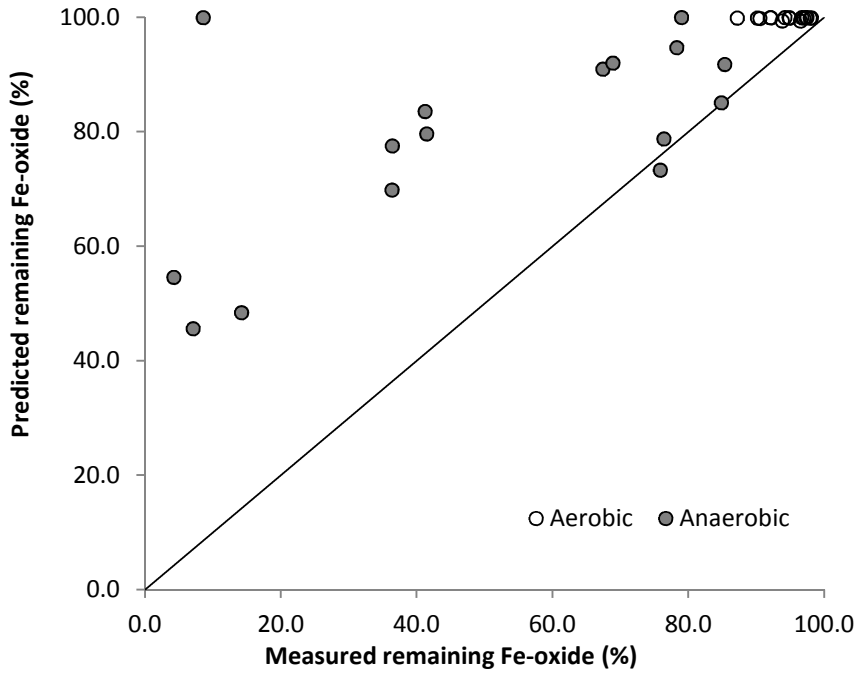

Fig. 5.22. Predicted versus measured (%) remaining Fe-oxide content under both aerobic and anaerobic conditions; predictions were made with WHAM(VI), parameterized by Case 3. The solid line represents the 1:1 relation.

Initially, the effect of reducing conditions was to increase the adsorption of Pb to Fe-oxide. This might be caused by strong re-adsorption on the remaining Fe- or Mn-oxides due to increase pH resulting from the developing anaerobism. Moreover, initial dissolution of both Mn- and Fe-oxide might increase the capacity for metal sorption on the remaining oxide surface (Contin et al., 2007). However, this adsorption process must then decline under more reducing condition resulting from two mechanisms: (i) reductive Mn- and Fe-oxides dissolution ultimately results in loss of adsorption capacity and (ii) competition from Fe^{2+} for metal binding sites. The current results suggest that these processes are active, notwithstanding the underestimation of Mn-oxide

dissolution predicted by WHAM(VI) (Case 5(Mn)). Fig. 5.23 shows that a binary trend exists for Pb binding to Fe-oxides as a function of $\%E_{resin}$(FeOx). Lead adsorption increased until ~ 20 % of Fe-oxide was dissolved (grey symbols; Fig. 5.23) and thereafter Pb adsorption decreased over the course of continuous of reductive dissolution of Fe-oxides (open symbols; Fig. 5.24).

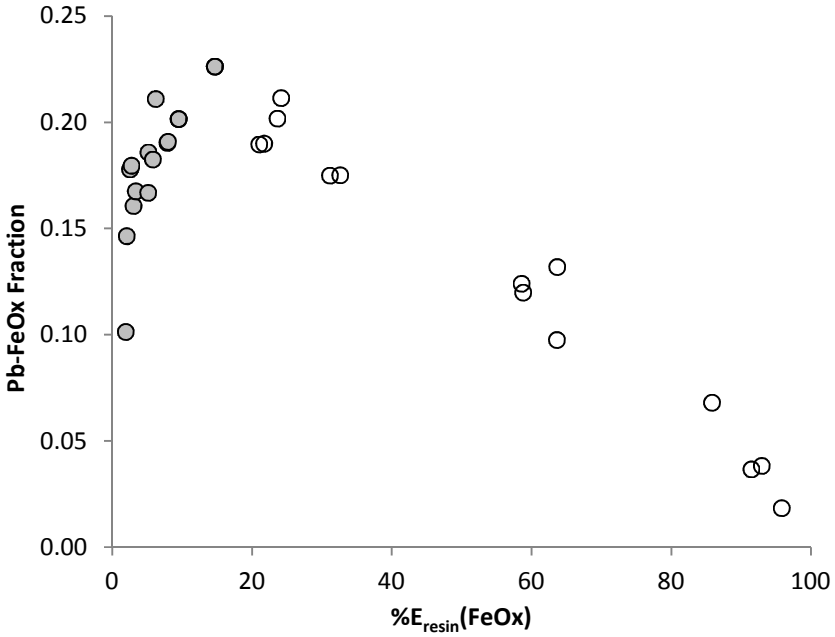

Fig. 5.23 Lead associated with Fe-oxides as a function of Fe $\%E_{resin}$ (FeOx); the grey and open symbols differentiate between the two conflicting trends (see text for more details).

Using the isotopically exchangeable pool of Fe as an input variable to WHAM clearly demonstrates the dual role of Fe as a competitor ion and as an adsorption phase for Zn, Cd and Pb in predicting metal solubility. An alternative to measuring isotopically exchangeable Fe (and Mn if possible) is to first predict adsorbed (labile) Fe^{II} and Mn^{II} from measured solution concentrations, using WHAM, and then iteratively estimate loss of reactive Fe and Mn oxide content. Currently, however, WHAM appears to underestimate adsorbed labile Fe and Mn using this approach. Furthermore, because WHAM(VI) also overestimates dissolved Pb then it follows that, if the underestimation of Mn and Fe oxide

dissolution were corrected, then this would further increase the error in predicting Pb solubility. More soils need to be investigated, with varied characteristics, to clarify the importance of Fe and Mn oxides as sources of adsorption sites and competitor ions during progressive reduction.

6. Chapter 6: Conclusion

6.1 Overview

The current work investigated isotopic methods as a means of assessing the labile metal pool in soils. The thesis set out to establish a pragmatic analytical method for the determination of the labile metal pool of Fe, Zn, Cd and Pb in contaminated soils. This was achieved by developing a multi-element stable isotope dilution method for simultaneously measurement of Fe, Zn, Cd and Pb lability in soils and comparing the results with commonly used extraction approaches such as single and sequential extractions. The thesis comprises four themes: (i) developing the isotopic method to maximise accuracy and precision in the determination of E-value while minimising cost and maximising efficiency of the assay (Chapter 2 & 3), (ii) validating the developed methods for a sub-set of soils collected from two old Pb/Zn mining areas (Black Rocks and Clough Wood sites, Derbyshire, UK) and comparing the isotopic results with commonly used extraction approaches (Chapter 3), (iii) assessing Zn, Cd and Pb dynamics on a large (catchment-wide) scale using 245 soil samples collected from a partially contaminated catchment (Weardale, North Pennines, UK) and using simple soil characteristics to predict metal isotopic exchangeability and the geochemical speciation model WHAM(VI) to predict metal solubility (Chapter 4) and (iv) determining the effect of oxidation - reduction fluctuation on metal lability and solubility as well as utilizing ^{57}Fe, possibly for the first time, to illustrate the dual role of Fe as a competitor ion and as an adsorption phase for Zn, Cd and Pb in solubility predictions using WHAM(VI) (Chapter 5).

6.2 Main findings

Multi-element stable isotope dilution can simultaneously assay metal lability for Zn, Cd, Pb and Fe in contaminated soils. Following several trials to optimize the instrumental and experimental conditions, a test of Fe^{II}, Zn, Cd and Pb recovery using suspensions of Amberlite IR 120 showed average % lability values of 97.8,

100, 97.8 and 98.7 respectively. Recovery was consistent over a range of spike concentrations.

The optimized approach to measuring isotopic exchangeability was based on a number of criteria. The stable isotope with the lowest natural abundance was used, where possible, to minimise the spike concentration required to produce a *quantifiable* change in isotope ratio. This was judged to constitute ~ 10% of the native ***labile*** metal pool of ^{70}Zn, ^{108}Cd and ^{204}Pb isotopes in the soil. The advantage of minimising the addition of spike isotope is that the measurement of E-value is then less likely to affect the result. Isotopic abundance was determined only for the 'major' and the spiked isotopes, for Zn and Cd, with the other isotopes assayed by inference from their natural abundance. This approach effectively minimizes the effect of ICP-MS plasma noise interferences. Thus the isotope pairs determined were ^{66}Zn and^{70}Zn, ^{108}Cd and^{111}Cd; all four Pb isotopes had to be measured because of the mixed radiogenic sources present in soils. It was found to be necessary to use an isotope immediately below the lighter measured isotope in the measurement sequence to allow the quadrupole to 'locally stabilise'. Thus ^{63}Cu, ^{107}Ag and ^{202}Hg were measured immediately before the isotopes ^{64}Zn, ^{108}Cd and ^{204}Pb. Isobaric interferences were also assessed, such as ^{108}Pd, ^{35}Cl-^{35}Cl and ^{204}Hg with ^{108}Cd, ^{70}Zn and ^{204}Pb respectively by comparison between standard and CCT-KED modes of ICP-MS operation. The chlorine dimer was found to substantially affect assay of ^{70}Zn and CCT-KED mode was adopted as standard in all work. Interference from 'non-labile colloidal' (NLC) metal was assessed by comparison between assay of E-value in the solution and resin phases, following isolation of filtered soil suspension supernatant solutions. Attention to all these potential sources of error increased the accuracy, precisely and reducibility of the assay and reduced the overall experimental cost so that each sample, run with 4 replicates, cost less than £0.15 (excluding the ordinary running cost of the ICP-MS).

For some soils, the presence of NLC metals in the solution phase could lead to *small but si*gnificant overestimation of E-value; these were mainly soils with high organic matter and/or carbonate contents. Therefore, a 'purification' step, with Chelex-100, is recommended for routine use with ID procedures in such cases. However, E-values measured in the solution phase are normally sufficient as an acceptable estimation of the labile metal pool where the effect of the NLC metals is likely to be low.

Assuming that E-values represent the best possible estimate of a 'reactive' metal pool, it is worth assessing simple extraction methods as an alternative approach to evaluating metal lability. This comparison appeared to depend on the soil characteristics, the origin of the metal contamination and the extractant used. For instance, using 0.05 M EDTA and 0.43 M HNO_3 in organic soils with low pH appears justifiable; E-values and 'extractable' metal assays were similar. However, the traditional DTPA extraction protocol fell below the E-value in most cases as its extraction capacity was limited, especially in acidic organic soils.

In addition, E-values also showed no ***exact*** match with individual SEP fractions or combinations of fractions. There was reasonable correspondence with metal extracted in the combined fractions F1+F2 of the Tessier SEP for Zn and Cd in all soils tested and in F1 and combined F1-F3 of the SEP for Pb in calcareous minespoil and acidic organic soils, respectively.

Metal lability was clearly dependent on a combination of 'source characteristics' and soil properties, principally pH, humus content and total metal concentration.

E-values showed a very wide range across the 245 soil samples collected from the Weardale catchment; % E-values were almost 1% - 100% for Zn, Cd and Pb depending on the history of contact between minespoil and soil materials and on the prevailing soil conditions. However the range of E-values also showed clear differences between the three metals; relative lability followed the, not entirely predictable, sequence Cd > Pb > Zn.

Soil characteristics were used to predict the metal lability using a multiple regression approach; approximately 80%, 53% and 66% of the variability in the isotopically exchangeable pool of Zn, Cd and Pb, respectively, were explained by the variation in soil pH, %LOI and total metal content. However, it was necessary to account for a binary trend in %E-value with ***increasing*** soil metal content thought to arise from (i) increasing lability due to occupancy of progressively weaker adsorption sites and (ii) descreasing lability in the presence of inert/non-labile metal from primary and secondary minespoil mineral at higher metal concentrations.

Including the isotopically exchangeable metal pool greatly improved the prediction of solubility in geochemical models over total metal concentration. Using total metal concentration as input to WHAM(VI), generally overestimated the total soluble metal concentrations for the three metals, particularly in the case of Pb. The best model predictions were for Zn when mainly associated with humic substances and present at relatively large concentrations in the solution phase under low soil pH conditions. The model generally overestimated the total soluble Pb. Unexpectedly, it appeared that poor prediction of soluble Pb may be due to strong binding to soil organic matter. Therefore, the optimizing the 'active soil organic matter' content as an input to WHAM 6.0 could possibly improve the model deviation.

The solubility and lability of Zn, Cd, Pb and Fe were followed under fluctuating redox conditions using soil suspensions aerated with air or nitrogen. Increasing solubility of Zn, Cd and Pb was seen during the oxidising phase of alternating redox incubation, and of Zn and Cd under continuously oxidising conditions. This was largely explained as being due to changes in soil pH. However, increasing Pb solubility was also likely to be affected significantly by higher DOC concentrations under continuous reducing conditions as it is well recognised that Pb is more strongly bound to soluble humus ligands.

Isotopically exchangeable ferrous Fe was successfully measured simultaneously with Zn, Cd and Pb. The redox incubation experiment showed that the proportion of Fe^{2+} measured in the solution phase was very small compared to the adsorbed labile pool of Fe^{2+}; around 95% of the reactive Fe^{2+} was adsorbed on soil constituents rather than dissolved in the solution phase. Traditionally, Fe^{2+} measured in the solution phase has been used as a key indication of reductive Fe-oxide dissolution but with no knowledge of the extent of Fe^{III}-oxide dissolution.

The reducible iron oxide content, isotopically exchangeable pool of Fe^{II}, measured Fe in solution and calculated Fe^{3+} activity were used as input variables to WHAM(VI), employing both *constant* and *variable* Fe-oxide contents, to evaluate the dual role of Fe as a competitor ion and as an adsorption phase for Zn, Cd and Pb in predicting metal solubility. Results for Zn, Cd and Pb showed that predicted values of solution concentrations were generally within one log unit of measured values. As expected, the greatest effects arising from variable Fe inputs to WHAM (eg Fe concentration in solution and variable Fe oxide content) were observed under reducing conditions. Generally, Zn and Cd were affected only by Fe^{2+} competition for adsorption sites because more than 90% of both metals were associated with organic matter and so fractionation was not affected by variation in Fe-oxide content. However, results for Pb solubility showed that competition from Fe^{2+} for adsorption sites was less important than loss of Fe oxide sites with progressive anaerobism due to greater association with Fe oxides. A substantial Pb fraction was also associated with Mn-oxides and so it was considered necessary to try and account for loss of adsorption sites on Mn-oxides under anaerobic conditions. In the absence of a measured value for isotopically exchangeable Mn^{2+} an attempt was made to use the WHAM-predicted reactive Mn^{II} from measured solution concentrations and then iteratively estimate loss of reactive Mn oxide content. Currently, however, from comparison of measured and WHAM-predicted labile Fe^{II}, it appears that the

model underestimates adsorbed labile Fe (and, by inference, Mn) using this approach. Furthermore, because WHAM(VI) also overestimates dissolved Pb then it follows that, if the underestimation of Mn and Fe oxide dissolution were corrected, then this would further increase the error in predicting Pb solubility.

6.3 Future work

Recently, increasing access to inductively coupled plasma mass spectrometry (ICP-MS) has led to greater use of stable isotope dilution applied to a wide range of elements. Potentially the method can be utilised for any element that has more than one stable isotope (about 60 elements, Ayoub, 2000) or long-lived radioactive isotopes (e.g. ^{129}I, Shetaya et al., 2011).

The current work has confirmed the viability of rapid simultaneous determination of multiple isotopically exchangeable metal pools in soils. This approach requires neither special safety precautions, as required in case of radioactive isotopes, nor labour intensive procedures, as required in sequential extraction methods. Potentially, isotopic dilution approaches to determine the labile metal pool in soils could replace traditional extraction methods and might be used in future as a routine laboratory procedure – perhaps even in regulatory measurements but certainly in research contexts that involve geochemical modelling.

Stable isotope approaches also facilitate investigation of the dynamics of elements in nano-particulate associations. The current study showed that stable isotopes can be successfully used to determine the proportion of metal that is associated with sub-micron colloidal particulates. This technique could provide a powerful tool for environmental nano-material risk assessment and studies of the kinetics of nano-particulate weathering etc. In this context, combining hyphenated chromatography approaches with stable isotopic dilution could confirm the existence of nano-particulate forms of non-labile metal in solution/suspension, as suggested from the comparison of E_{resin} and E_{soln} in Chapter 3 and 4. One form that such an investigation could take would be a

simple comparison of the molecular weight distribution of metal in soil solutions using size-exclusion chromatography linked to ICP-MS. Differences in metal distribution between spiked and non-spiked soil solutions could indicate the existence of non-isotopically mixed metal fractions – perhaps with less representation of the spike in the high molecular weight fraction, for example.

Further work is required to investigate the definitive roles of Fe in reducing environments and fluctuating redox conditions, using stable iron isotopes to quantify the extent of Fe oxide reduction and subsequent ferrous iron (Fe^{2+}) adsorption as a labile cation. Only a single soil was investigated over a comparatively short time period in the current study. It would be particularly interesting to couple more extended studies to simulations of the 'whole soil' behaviour of metals under reducing conditions using the new version of WHAM(VII).

Using DGT in combination with stable isotope can offer a straightforward measurement of labile metal concentration in the Chelex-100 layer of the DGT device, beside the ordinary measurement of the time-integrated concentrations of labile metal species provided by DGT. Some time-dependent work with DGT applied to isotopically labelled soil was undertaken during the course of this project but the results are not presented here.

Isotopic dilution alone only provides sufficient information about the total labile metal pool in soils. However, coupling the isotopic dilution technique with advanced spectroscopic techniques (eg EXAFS) could indicate the precise metal-ligand conformations associated with the 'labile' and 'non-labile' forms of the metal. Few studies have previously undertaken such a comparison, with mixed results (Sarret et al., 2004, Ahmed et al., 2009, Degryse et al., 2011). We know that isotopically exchangeable metal is not simply adsorbed by electrostatic forces alone ('*cation-exchangeable*'), but the precise identity of labile adsorbed and non-labile species remains unknown.

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